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Observations Concerning the Toxicity, Absorption and Therapeutic Effect of Sulphanilamide and Certain Related Organic Sulphur-containing Compounds in Experimental Infections in Mice. W. Harry Feinstone, B.S., Eleanor A. Bliss, Sc.D., Earl Ott and Perrin H. Long, M.D.. 565

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OBSERVATIONS CONCERNING THE TOXICITY, ABSORPTION AND THERAPEUTIC EFFECT OF SULPHANILAMIDE AND CERTAIN RELATED ORGANIC SULPHUR-CONTAINING COMPOUNDS IN EXPERIMENTAL INFECTIONS IN MICE[1]

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From the Biological Division of the Department of Medicine, in the Johns Hopkins University, Baltimore, Maryland

Submitted for publication March 1, 1938

The introduction of "Prontosil," "Prontosil Solution"[2] and Sulphanilamide (1, 2, 3, 4, 5, 6) as therapeutic agents for the treatment of certain bacterial diseases has brought a renaissance in the study of chemotherapy. A natural result of the successful use of these original compounds has been the search—one might almost say the race—for new effective chemotherapeutic compounds. Throughout the world chemists are engaged in synthesizing new sulphur-containing compounds. Hardly a month passes without new attributes being reported for the original compounds and the list of new derivatives is constantly increasing. Rapid advances have been made during the past year towards a better understanding of the original compounds.

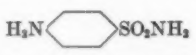
The very nature of these advances, however, calls for the sober recognition of the fact that great care must be taken in evaluating the therapeutic and toxic effects of new chemotherapeutic agents. The recent reports of toxic effects of sulphanilamide in human beings definitely show that acute or chronic toxicity tests made with sulphur-containing compounds in animals are of little value in predicting the behaviour of these compounds in man.

[1] This investigation has been made possible by a grant from the Chemical Foundation, Inc. of New York City.

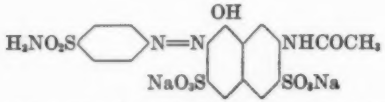
[2] "Prontosil Solution" is now marketed in the United States under the name of "neoprontosil," by the Winthrop Chemical Co., Inc.

Nevertheless, it is becoming increasingly important that all new chemotherapeutic agents be studied adequately in experimental

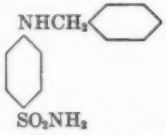
CHART I. THE CHEMICAL STRUCTURE OF SULPHANILAMIDE AND CERTAIN RELATED COMPOUNDS



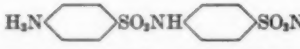
Para-aminobenzene-sulphonamide (sulphanilamide)



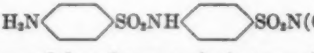
Disodium 4′-sulphamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphthalene-3,6-disulphonate ("Prontosil Soluble")



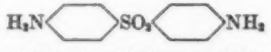
Para benzylamino-benzene sulphonamide ("Setazine," "Septazine")



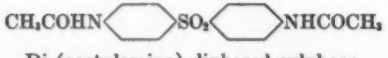
Para-amino-benzene-sulphonyl-para-aminobenzene-sulphonamide ("disulphanilamide," "Disulon")



Para-aminobenzene-sulphonyl-para-aminobenzene-dimethylsulphonamide (Dimethyl derivative of disulphanilamide, "D.B. 90," "Uliron")



4:4′ Diamino diphenyl sulphone



Di (acetylamino) diphenyl sulphone

animals before being introduced for clinical trials. While it may be impossible to predict toxic effects in human subjects from tests in

laboratory animals, still there are many problems which may be clarified by animal experimentation. Among these are the relation of the absorption of a compound to its acute toxicity, the blood levels obtained following the administration of a compound, the disposal of the administered compound, and the breakdown or conjugation of the compound. Relative therapeutic efficiencies of various compounds can be determined only in experimental infections in statistically significant numbers of laboratory animals. Only if adequate laboratory studies are made can one be spared the spectacle of evaluations of therapeutic efficiencies of new compounds which have been determined on statistically insignificant numbers of animals, of claims of non-toxicity for compounds which may be almost completely unabsorbed, and of computations of the therapeutic-toxic indices of new compounds, when actually no real information has been made available on either of the two components of the index.

It will be our purpose in this communication to outline certain minimal requirements necessary for the proper evaluation of old or new sulphur-bearing compounds. We will present data regarding the rate of absorption, comparative toxicity and therapeutic efficiency of sulphanilamide, "Prontosil Solution," para amino benzene sulphonyl-sulphanilamide ("disulphanilamide," "Disulon"), the dimethyl derivative of "disulphanilamide" ("D.B. 90," "Uliron") (these compounds were supplied through the courtesy of Dr. O. W. Barlow of the Winthrop Chemical Company), para benzyl amino benzene sulphonamide ("Setazine," "Septazine") (Merck and Company, Inc.) and 4:4′ diamino diphenyl sulphone and di (acetyl amino) diphenyl sulphone. (These latter two compounds were prepared by Dr. George W. M. Raiziss of the Dermatological Research Laboratories.) The chemical structure of these compounds is shown in Chart I.

METHODS

Adult white mice of both sexes weighing from 17 to 25 grams each were used in our experiments.

Toxicity Tests: The individual mice were accurately weighed and the dosage of each compound was computed upon the basis of grams per kilogram of mouse body weight. All of the tests were made by the oral route, the compounds having been suspended in 10 per cent solutions of gum acacia. The acacia solutions were

prepared by adding 10 grams of powdered acacia to 100 c.c. of tap water and then heating in a steam bath until a smooth mucilaginous solution was obtained. The compounds to be tested, if not already finely powdered, were ground in a mortar until the required fineness was secured. The suspensions, after vigorous shaking, were administered to the mice perorally by injecting the required dose directly into the stomach, through a blunt No. 17 gauge needle attached to a 2 c.c. syringe. The mortality among the mice resulting from accidents during the administration of the compound was about 1 per 1,000 peroral injections. All mice used for toxicity tests were observed for a period of three days, and all deaths occurring within that period were considered to be due to the toxic effects of the administered compound. In the presentation of the data derived from these determinations, the tests at all levels of a given compound up to the first toxic level have been grouped together. After that each level is presented separately.

Blood Level Determinations of the Various Compounds: In determining the blood levels obtained after the administration of various amounts of the different compounds, the methods of sulphanilamide determination described by Marshall and his associates (7) were used. In determining the blood levels of sulphanilamide, "Prontosil Solution" (in terms of free sulphanilamide), the dimethyl derivative of disulphanilamide and diamino diphenyl sulphone, the toluene sulphonic acid method was used in precipitating the blood. In determining the blood levels of disulphanilamide, the alcohol precipitation method gave more accurate estimations than the toluene sulphonic acid method. It was noted with disulphanilamide that at least 20 minutes had to be allowed before precipitation became complete, and with diamino diphenyl sulphone that the colorimetric readings had to be made within five minutes after coupling because otherwise the standard would rapidly increase in color and the readings would be variable. In all of these determinations the compound under test was used as the standard. The accuracy of these methods was established by the determination of the compounds in bloods to which known amounts of the various chemicals had been added.

The blood levels obtained from either a single parenteral or peroral administration of the various compounds after one hour were determined as were also the levels over varying periods of time. The blood levels following maximum absorption from a single peroral or subcutaneous administration were determined for sulphanilamide, "Prontosil Solution" (in terms of free sulphanilamide), disulphanilamide, the dimethyl derivative of disulphanilamide and diamino diphenyl sulphone. The blood levels of sulphanilamide alone were determined following the thrice daily peroral administration of 6 milligrams of sulphanilamide over a period of six days. In this instance the blood was obtained four hours after the daily morning dose of sulphanilamide.

Therapeutic Use of the Various Compounds in Experimental Infections in Mice: Hemolytic Streptococcal Infections. C2O3, the well known mouse-virulent strain of the beta hemolytic streptococcus was used for producing experimental hemolytic

streptococcal infections in mice. Twelve to fourteen hour dextrose blood broth cultures, incubated at 37°C. and derived from the heart's blood of a fresh passage mouse, were diluted 1–500,000 in beef infusion broth and 0.5 c.c. of this dilution represented the infecting dose. A long experience with this strain shows that such an inoculum contains from 100 to 1000 M.L.D. All inoculations were made into the peritoneal cavities of the mice. Three types of therapy were employed in experimental hemolytic streptoccal infections in mice. In the type A experiments the mice were infected in the routine manner, treated immediately and twice thereafter on successive days, by the parenteral route. Type B experiments were carried out in the same manner, except that the treatments were given *per os*. In the type D experiments, four hours were permitted to elapse before treatment was begun, then the mice were treated *per os* t.i.d. for five days, twice on the sixth day and once on the seventh. The mice in the type A and B experiments were observed for 14 days, and those in the type D experiments for 30 days. Cultures upon the surface of rabbits' blood agar plates were made from the heart's blood of all of the mice dying within the periods of observations, in order to determine whether or not they had died of hemolytic streptococcal infections. In mice treated by the parenteral route, the therapeutic compounds were dissolved in physiological saline, while in those treated *per os* the compounds were suspended in 10 per cent acacia solution.

Pneumococcal Infections. Strains of type I and type II pneumococci, which were obtained from Dr. Lloyd Felton and which were highly virulent for mice, were used in all of these experiments. Cultures obtained from the heart's blood of a passage mouse were grown in rabbits' blood broth for twelve to fourteen hours at 37°C. They were diluted to 1–5,000,000 and 0.5 c.c. was injected by the intraperitoneal route into mice. This represented 100 M.L.D. of each mouse-virulent strain of pneumococcus. Treatment *per os* with the various compounds was instituted immediately after infection and was continued t.i.d. for five days, twice on the sixth day and once on the seventh day. All surviving mice were observed for thirty days. Heart's blood cultures upon the surface of blood agar plates were made from all mice dying within the period of observation in order to determine the cause of death.

Meningococcal Infections. Strains of types I and II meningococci were obtained from the National Institute of Health, Washington, D. C., through the kindness of Dr. Sara E. Branham. Both strains were, in the presence of mucin, highly virulent for mice. The type II strain was used in the first three experiments and the type I in the last five. As there was little difference in the virulence of these two strains and no difference in the therapeutic effects of the different compounds in combating infections due to them, they have been treated as one strain in compiling the data. Mice were inoculated intraperitoneally with 0.5 c.c. of a 1 to 50 mucin dilution of a saline suspension of the growth from an 8 hour blood agar slant (8). The saline suspension was prepared by washing the growth from the slant with 2 c.c. of physiological salt solution and diluting this

as quickly as possible until it had approximately the same degree of turbidity as an arbitrary standard made of finely divided pyrex glass suspended in distilled water (9). A microscopic count of one "standard" saline suspension of meningococci made in this way showed 250 million organisms (single or diplococci) per cubic centimeter, so that the infecting dose was approximately 2.5 million meningococci. However, as the suspensions were standardized rather roughly, virulence controls at 1–50,000 and 1–500,000 dilution in mucin were included in each test. These showed that the infecting dose contained 10,000 M.L.D. The mice were given a single treatment immediately after they had been inoculated with the meningococci. The compounds were suspended in 10 per cent acacia and administered by mouth. The animals were kept under observation for 14 days and cultures were made from the heart's blood of those that died in this period.

Staphylococcal Infections. Two strains of staphylococci were used; one was obtained from Dr. O. W. Barlow of the Winthrop Chemical Company, Rennselaer, New York; the other, a haemolytic staphylococcus aureus, was isolated at the Johns Hopkins Hospital from the sputum of a patient suffering from staphylococcal pneumonia. Neither strain was very virulent for mice but Dr. Barlow's suggestion (10) that mice could be infected with his strain if they were given, intravenously, 0.2 c.c. of a heavy suspension, was carried out with considerable success, with both strains. (Ninety per cent of the control animals died. The most striking findings at autopsy were multiple abscesses in the kidneys.) The broth suspensions were made by washing the growth from a blood agar slant incubated for 16 hours with 6 c.c. of beef infusion broth. The mice were injected in one of the tail veins. Fifteen to thirty minutes after they had been infected they were given their first treatment, by the oral route, with acacia suspensions of the different compounds. Treatments were repeated twice daily for the first five days and once a day for the next five days. The mice were observed for thirty days and any that died were autopsied. If no abscesses were found, the kidneys were cut open aseptically, and cultures were made by rubbing a loop on the cut surface and transferring the exudate to blood agar.

RESULTS

Acute Toxicity Tests. Our observations regarding the toxicity, for white mice, of sulphanilamide and certain allied compounds, are given in Table I. It will be noted that our results concerning the toxicity of sulphanilamide are in accord with those of Dyke (11) and of Marshall *et al.* (12), but are somewhat lower than the toxicity figures as reported for this compound by Buttle and his associates (13), Rosenthal (14), Halpern and Mayer (15) and Barlow (16). Toxicity tests performed with "Prontosil Soluble," "disulphanilamide" and the dimethyl derivative of "disulphanilamide" gave results

comparable to those reported by Barlow (16) and, in so far as "disulphanilamide" was concerned, to those reported by Rosenthal *et al.* (14).

Para benzyl amino benzene sulphonamide was relatively non-toxic in our experience. 4:4′ diamino diphenyl sulphone was very toxic and our data on this point show about the same degree of toxicity as did the data of Buttle *et al.* (17). Para di (acetyl amino) benzene sulphone was essentially non-toxic in mice.

TABLE I

The acute toxicity of sulphanilamide and certain related compounds in mice

COMPOUND	DOSE	NUMBER OF MICE	NUMBER DEAD	PER CENT DEAD
	gms./kilo mouse			
Sulphanilamide	1.0–3.0	25	0	0
	3.5	15	12	80
	4.0	50	46	92
"Prontosil Soluble"	1.5–12.0	30	0	0
p-Benzylamino benzene sulphonamide	8.0–20.0	40	0	0
Disulphanilamide	8.0–20.0	30	0	0
Dimethyl derivative of Disulphanilamide	20.0	20	0	0
4:4′ Diamino diphenyl sulphone	0.1	25	0	0
	0.2	25	7	28
	0.3	25	14	56
	0.35	25	12	48
	0.4	25	13	52
	0.45	25	13	52
	0.55	25	15	60
	0.65	15	15	100
Di(acetyl amino) diphenyl sulphone	10.0	15	1	6.6
	20.0	15	0	0

Blood Levels of Sulphanilamide and Related Compounds Following the Administration of a Single Dose in Mice. In Table II data are presented concerning the blood levels obtained after varying periods of time, following the administration of a single dose of sulphanilamide or certain related compounds.

These values were obtained by pooling the blood of 4 or 5 mice for each given point. It is to be noted that, in general, the highest values are obtained at the end of one hour, regardless of the route of administration of the compounds. "Disulphanilamide" in 1 or 2

TABLE II

Blood levels of sulphanilamide and certain related compounds following single doses in mice

COMPOUND	DOSE	NUMBER OF MICE PER POINT	BLOOD LEVELS IN MGMS. PER CENT HOURS FOLLOWING THE ADMINISTRATION OF THE COMPOUND																			
			1		2		3		4		5		6		7		8		9		10		24	
			F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C	F	C
Sulphanilamide	10 mgms. p.o.	4	20.0	0	10.9	0	8.2	1.8	5.6	3.4			2.1	1.9			1.0	1.2			T	T		
Sulphanilamide	10 mgms. s.c.	4	37.8	0.02	20.8	0	10.0	1.8	5.6	1.8			1.8	1.5			0.9	1.0			T	T		
Sulphanilamide	6 mgms. s.c.	5	18.4	0	9.0	0			6.8	1.3			1.5	1.6					0.9	0.9				
Sulphanilamide	1 gm./k. p.o.	5	76.2	3.8			46.5	0.5																
Sulphanilamide	2 gms./k. p.o.	5	126.2	2.3			44.3	2.6																
Sulphanilamide	3 gms./k. p.o.	5	188.0	12.1			76.4	7.0	34.7								7.4						10.3	
Sulphanilamide	4 gms./k. p.o.	5	185.0	16.7			77.0	8.0																
"Prontosil Solution"	6 mgms. s.c.	5	0.1	0.5	0.6	0.6			1.3	0.7			1.3	1.4					0.2	0.9				
"Prontosil Solution"	20.5 mgms. s.c.	5	1.4	0.8	1.7	0.8			4.4	1.0			3.0	1.0					0.1	1.3				
Disulphanilamide	10 mgms. p.o.	5	2.1		2.5				1.6															
Disulphanilamide	0.25 gm./k. p.o.	5	2.4		2.1				1.5															
Disulphanilamide	1 gm./k. p.o.	5	3.5		4.1				3.7															
Disulphanilamide	2 gms./k. p.o.	5	3.9		4.4				4.7															
Disulphanilamide	3 gms./k. p.o.	5	3.2		2.4				3.6				2.7				1.9				1.2		0.9	
Dimethyl Derivative of Disulphanilamide	10 mgms. p.o.	5	3.4		3.0		2.3		2.0		1.8				1.6									
4:4′ diamino diphenyl sulphone	0.75 mgm. p.o.	5	2.8		1.9				1.4				0.8											
4:4′ diamino diphenyl sulphone	1.25 mgms. p.o.	5	3.6		3.3				1.7				2.0											

F = sulphanilamide.
C = Conjugated sulphanilamide.
p.o. = per os.
s.c. = subcutaneous.

gram doses showed highest levels in from 2 to 4 hours. The parenteral injection of sulphanilamide gave higher immediate blood levels of this compound than did corresponding perorally administered doses. Sulphanilamide alone, of all the compounds tested, gave increasingly higher blood levels up to 3 grams per kilogram of mouse body weight. The sublethal dose of sulphanilamide (3 grams per kilogram) gave essentially the same sulphanilamide blood level at the end of one hour as did the lethal dose (4 grams per kilogram). The fall in the blood level of sulphanilamide following a single parenteral or peroral dose of the drug was very rapid except when a large dose was given by the peroral route.

The peroral administration of "disulphanilamide" or its dimethyl derivative was followed by low blood levels of these compounds and with "disulphanilamide" there was no increase in the blood level

TABLE III

The blood level at the end of one hour following increasing doses of 4:4′ diamino diphenyl sulphone in mice

	AMOUNT OF DRUG PER OS IN MILLIGRAMS									
	0.75	1.25	2.5	4	5	6	7	8	9	10
Blood level, mgms. %	2.8	3.5	5.2	8.6	8.9	8.3	7.1	9.0	7.7	9.1

of the compound when doses of more than one gram per kilogram of mouse body weight were used. Mice receiving a large dose of "disulphanilamide" showed a slow fall in the blood level of the compound over a period of 24 hours.

Small doses of 4:4′ diamino diphenyl sulphone gave fairly high blood levels of the compound, but as is shown in Table III, increasing doses of this sulphone did not result in increased blood levels of the compound. Mice receiving 4 or more milligrams of the compound per mouse had essentially the same blood levels at the end of one hour.

The accuracy of the data derived from the pooled bloods of mice is shown by the observations contained in Table IV.

Here it is shown that determinations made upon the heart's blood of single mice one hour after the administration of sulphanil-

TABLE IV

The blood levels of sulphanilamide and certain related compounds attained in the blood of mice one hour after the administration of the following doses of the compounds

COMPOUND	DOSE	MOUSE										REMARKS
		1	2	3	4	5	6	7	8	9	10	
	mgms.											
Sulphanilamide	10 p.o.	40.0	31.8	28.2	40.0	32.2	38.9	28.3	64.5	22.6	23.9	Acacia suspension
	10 p.o.	21.5	21.9	23.7	28.6	25.4	25.1	24.4	22.4	23.3	17.2	Saline solution
	10 s.c.	48.4	40.0	42.5	50.0	50.2	48.9	51.0	50.2	56.8	51.2	Saline solution
	20 s.c.	80.8	55.7	88.6	87.1	90.1						Saline solution
	10 p.o.	38.6	31.2	28.7	32.1	29.9	30.8	34.1	26.1	30.0	24.8	Acacia suspension. Mice infected with Hemolytic streptococci
Disulphanilamide	10 p.o.	3.10	3.80	3.2	3.3	3.8	4.2	3.1	3.6	3.0	3.7	Acacia suspension
Dimethyl, Derivative of Disulphanilamide	10 p.o.	3.9	5.9	4.7	2.8	2.8	2.9	4.3	4.5			Acacia suspension
4:4′ Diamino diphenyl sulphone	1.25 p.o.	3.8	3.2	3.5	3.0	3.7	3.8	3.1	3.6	3.3	3.6	Acacia suspension

p.o. = per os.
s.c. = subcutaneous.

amide or related compounds give single figures that are in harmony with those obtained from the pooled bloods of a number of mice.

It will be noted in Table II that following the parenteral injection of "Prontosil Solution" into mice, free sulphanilamide can be determined at the end of one hour and that the amount of sulphanilamide found in the blood increases as the dose of "Prontosil Solution" is increased. An especially interesting observation was that if the molecular equivalent of 6 milligrams of sulphanilamide in terms of "Prontosil Soluble" (20.5 mgms.) was injected parenterally into mice,

TABLE V

The level of measurable sulphanilamide in the blood of mice four hours after the administration of "prontosil soluble" and sulphanilamide by the parenteral route

MOUSE	20.5 MGMS. "PRONTOSIL SOLUBLE"	6 MGMS. "PRONTOSIL SOLUBLE"	6 MGMS. SULPHANILAMIDE
1	4.41	1.41	4.96
2	4.49	1.33	5.11
3	5.09	1.40	4.78
4	4.81	1.37	5.07
5	4.36	1.31	5.23

TABLE VI

The level of measurable sulphanilamide in the blood of mice four hours following increasing doses of "prontosil soluble" by the oral route

	AMOUNT OF DRUG PER OS IN MGMS. PER KG. OF MOUSE WEIGHT			
	1 gm. per kg.	2 gms. per kg.	3 gms. per kg.	4 gms. per kg.
Blood level, mgms. %, 4 hours.....	7.03	6.81	6.07	5.56

the blood levels of free sulphanilamide at the end of four hours were essentially those obtained at the same period following the parenteral injection of 6 milligrams of sulphanilamide. If, however, 6 milligrams of "Prontosil Soluble" were injected, the blood levels of free sulphanilamide were much lower than those obtained following the injection of 6 milligrams of sulphanilamide. The validity of these determinations upon the pooled bloods of mice is established by the observations of the blood levels obtained in single mice four hours after the injection of "Prontosil Solution" or sulphanilamide (Table V).

Another observation, shown in Table VI, was that the blood levels

of free sulphanilamide in mice were essentially the same four hours after the peroral administration of 1, 2, 3, or 4 grams of "Prontosil Soluble" per kilogram of mouse body weight.

We were unable to determine accurately the blood levels of para benzyl amino benzene sulphonamide or those of para di(acetyl-amino) benzene sulphonamide and for that reason we have not included data pertaining to those compounds in this part of this communication.

The Comparative Therapeutic Effects of Sulphanilamide and Certain Related Compounds in Experimental Infections in Mice Streptococcal Infections. In Table VII are shown the comparative therapeutic effects in mice treated by the parenteral route with 6 or 20.5 milligrams

TABLE VII

The therapeutic effect of brief parenteral treatment (type A) with sulphanilamide and its weight or molar equivalent of "prontosil soluble" upon experimental hemolytic streptococcal infections in mice

COMPOUND	NUMBER OF MICE	DEATHS FROM BETA HEMOLYTIC STREPTOCOCCAL INFECTIONS, DAYS												SURVIVALS	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	No.	%
Sulphanilamide 6 mgms.....	150		18	17	27	30	6	3	1	2		1	1			44	29
"Prontosil Soluble" Molecular Equivalent 20.5 mgms.....	100	7	6	6	19	14	9	2	1	2				1		33	33
"Prontosil Soluble" Weight Equivalent 6 mgms.....	100	29	22	12	15	6	1			1						14	14
Controls.....	566	538	25	2	1											0	0

of "Prontosil Soluble" once a day for three days and with 6 milligrams of sulphanilamide over the same period of time. It is to be noted that in the group of mice treated with sulphanilamide, no deaths occurred during the first day, while in the group treated with 20.5 milligrams of "Prontosil Soluble," 7 mice died in the first twenty-four hours. In both groups greater numbers of mice died after treatment had been discontinued than during the treatment period. If, on the other hand, mice are treated with 6 milligrams of "Prontosil Soluble" daily for three days, the majority of deaths take place during the period of treatment. At the end of 14 days, approximately the same percentage of survivors was found in the groups treated with 6 milligrams of sulphanilamide or 20.5 milligrams of "Prontosil

Soluble," while in the group of mice treated with 6 milligrams of "Prontosil Soluble" the percentage of survivors was about half of that of the first two groups.

There have been several reports (14, 17, 18, 19) dealing with the comparative therapeutic effects of sulphanilamide and related com-

TABLE VIII

The therapeutic effect of brief treatment (type B) with sulphanilamide and certain similar compounds upon experimental hemolytic streptococcal infections in mice

COMPOUND	NUMBER OF MICE	DOSAGE*	DEATHS FROM BETA HEMOLYTIC STREPTOCOCCAL INFECTION, DAYS														SURVIVALS	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	No.	%
Sulphanilamide	60	10.0 mg. QD 3	8	3	6	13	4	2	3								21	35
Disulphanilamide	50	10.0 mg. QD 3	3	7	3	19	5	3									10	25
Dimethyl derivative of Disulphanilamide	50	10.0 mg. QD 3	17	16	7	4	1										5	10
p-Benzylamino benzene sulphonamide	50	10.0 mg. QD 3	18	11	8	6	2	1				1					3	6
4:4′ diamino diphenyl sulphone	50	1.25 mg. QD 3	1	1	1												47	94
Di(acetyl amino) diphenyl sulphone	150	5.0 mg. QD 3		1	3	19	19	9	2	3	1	1	1		3	2	86	57.3
Controls	566		538	25	2	1											0	0

* See footnote table IX.

pounds in experimental hemolytic streptococcal infection in mice. Table VIII shows the therapeutic effect of brief treatment (Type B) with sulphanilamide and certain similar compounds upon the course of hemolytic streptococcal infections in mice. The results obtained with three daily doses of 10 milligrams of sulphanilamide are definitely superior to those obtained with the same dosage of "disulphanilamide,"

the dimethyl derivative of "disulphanilamide" and para benzyl amino benzene sulphonamide. 4:4′ diamino diphenyl sulphone in doses of 1.25 milligrams per day for three days gave superlative results. Treatment with 5 milligrams of di(acetylamino) diphenyl sulphone, while giving a lower percentage of survivors than the non-acetylated form, seemed superior to therapy with sulphanilamide and the other compounds used in this experiment.

It was considered advisable to test the therapeutic effects of these same compounds after the streptococcal infections had become established in mice. A broad experience with the hemolytic streptococcal strain C2O3 over the past four years has shown that, following an intraperitoneal injection of 100–1000 M.L.D. of this strain, the majority of the mice will have a positive tail blood culture within 4 hours. Accordingly, in the experiment shown in Table IX treatment of the mice was begun 4 hours after inoculation and was continued three times a day for 5 days, twice on the sixth and once on the seventh day. The mice were then observed for 30 days.

When the survival rate following prolonged treatment (Type D) is compared with the rate following brief treatment (Type B), some very interesting points are revealed. In the first place there is a marked improvement in the effect of sulphanilamide and a fair improvement in the effects of "disulphanilamide," its dimethyl derivative and para benzyl amino benzene sulphonamide. Di-(acetylamino) diphenyl sulphone which gave much better results than did sulphanilamide in the Type B experiments showed but a moderate degree of improvement when the Type D form of treatment was used. This was an entirely unexpected finding and is contrary to our previous experience. Little, if any, improvement in the effect of the 4:4′ diamino diphenyl sulphone was noted or expected. It was interesting, however, to note that the mice exhibited marked symptoms of acute toxicity as evidenced by a primary increase in activity, then staggering and finally in some instances temporary periods of coma, during the week of treatment with the 4:4′ diamino diphenyl sulphone.

Pneumococcal Infections. In these experiments, highly mouse virulent strains of Type I and II pneumococci were used and all therapy was given by the oral route. As will be seen in Table X,

TABLE IX

The therapeutic effect of prolonged treatment (type D) with sulphanilamide and certain similar compounds upon experimental hemolytic streptococcal infections in mice

COMPOUND	NUMBER OF MICE	DOSAGE*	DEATHS FROM BETA HEMOLYTIC STREPTCOCCAL INFECTION, DAYS															SURVIVALS	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 to 30	No.	%
Sulphanilamide	50	10.0 mg. TID 5 BID 1 QD 1				1	1										1	47	94
Disulphanilamide	50	10.0 mg. TID 5 BID 1 QD 1		2	1	2		2	4	4	1							34	68
Dimethyl derivative of Disulphanilamide	50	10.0 mg. TID 5 BID 1 QD 1			3	6	1	4	2	3		1	1	1			2	26	52
p-Benzylamino benzene sulphonamide	50	10.0 mg. TID 5 BID 1 QD 1			7	4	3		5	2	1		1		2	1	1	23	46
4:4′ diamino diphenyl sulphone	50	0.75 mg. TID 5 BID 1 QD 1		1	1													48	96
Di(acetyl amino) diphenyl sulphone	50	5.0 mg. TID 5 BID 1 QD 1					1		1	3	3	1	1		1		3	36	72
Controls	566		538	25	2	1												0	0

* In the dosage column in this table and in tables VIII, X and XII the figures indicate the size of the individual dose and the duration of treatment. For example, the 50 animals treated with sulphanilamide each received 10.0 mg. of the drug three times daily for 5 days (TID 5), twice daily for 1 day (BID 1), and once daily for 1 day (QD 1).

TABLE X

The therapeutic effect of prolonged peroral treatment with sulphanilamide and certain similar compounds in experimental pneumococcal infections in mice

COMPOUND	TYPE OF PNEUMO.	NUMBER OF MICE	DOSAGE*	DEATH, DAYS										SURVIVALS	
				1	2	3	4	5	6	7	8	9	10	10 to 30	No.	%
Sulphanilamide			10.0 mg.													
	I	25	TID 5	7	16	2									0	0
	II	50	BID 1	5	6	14	13	6	3	2	1				0	0
			QD 1													
Disulphanilamide			10.0 mg.													
	I	25	TID 5	22	3										0	0
	II	50	BID 1	32	13	3									2	4
			QD 1													
Dimethyl derivative of Disulphanilamide			10.0 mg.													
	I	25	TID 5	25											0	0
	II	50	BID 1	40	10										0	0
			QD 1													
p-Benzylamino benzene sulphonamide			10.0 mg.													
	I	15	TID 5	15											0	0
	II	30	BID 1	30											0	0
			QD 1													
4:4′ diamino diphenyl sulphone			0.75 mg.													
	I	61	TID 5	3	16	21	6	2	1						12	20
	II	122	BID 1	22	8	8	10	13	18	21					22	18
			QD 1													
Di(acetyl amino) diphenyl sulphone			5.0 mg.													
	I	25	TID 5	13	11		1								0	0
	II	50	BID 1	5	15	2	8	9	7	1		1			1	2
			QD 1													
Controls	I	50		50											0	0
	II	50		40	10										0	0

* See footnote table IX.

sulphanilamide seemed to have a slight effect in Type II experimental infections in mice. "Disulphanilamide," its dimethyl derivative and para benzyl amino benzene sulphonamide were essentially without

effect. Diamino diphenyl sulphone had a very definite effect in both the Type I and II infections in mice. In our experience this is the only chemotherapeutic agent which has shown an appreciable number of survivors at the end of thirty days. The survivors in this group were subsequently inoculated with 10–100 M.L.D. of the same strain of pneumococci and all succumbed promptly to a generalized pneumococcal infection. Di(acetylamino) diphenyl sulphone showed about the same degree of activity as did sulphanilamide in experimental pneumococcal infections in mice.

TABLE XI

The therapeutic effect of a single peroral dose of sulphanilamide and certain similar compounds upon experimental meningococcal infections in mice

COMPOUND	NUMBER OF MICE	DOSAGE	DEATHS, DAYS								DEATHS DUE TO MENINGOCOCCI*	PERCENTAGE DEATHS DUE TO MENINGOCOCCI*
			1	2	3	4	5	6	7	8 to 14		
		mgms.										
Sulphanilamide	50	10	10	3	2					4	6	12
Disulphanilamide	50	10	7	2	1						7	14
Dimethyl derivative of Disulphanilamide	50	10	11	3					1		13	26
p-Benzylamino benzene sulphonamide	50	10	21	3						2	23	46
4:4′ Diamino diphenyl sulphone	50	1.25	6	3						3	8	16
Di(acetylamino) diphenyl sulphone	50	5.0	10	2						2	10	20
Controls	50		50								48	96

* Meningococci were never recovered from mice dying after the second day, and seldom from those dying on the second day.

Meningococcal Infections. The therapeutic effect of sulphanilamide and certain related compounds in experimental meningococcal infections is shown in Table XI.

As will be noted, sulphanilamide, "disulphanilamide" and 4:4′ diamino diphenyl sulphone were all about equally effective in this type of experimental infection. The other three compounds under test showed moderate degrees of therapeutic efficiency.

It is our impression, however, that these results do not reflect

accurately the comparative therapeutic activity of these compounds in experimental meningococcal infections in mice. In the first place, only about a third of the mice treated with sulphanilamide which succumbed, showed meningococci in their heart's blood cultures. This would indicate that death was due to a toxic process rather than to an active bacteremia. It was noted that within an hour of inoculation all of the mice, regardless of the type of therapy used, appeared ill and generally remained so for at least twenty-four hours. It is known (20) that the intraperitoneal injections of dead meningococci may kill mice, and a somewhat similar phenomenon may have been operative in the experimental infections which we observed. It seems probable that the drugs were successful in combating the infection in the majority of the mice, but were powerless in many instances against the toxic process. The late deaths, occurring after the seventh day, were all due to salmonella infection.

Staphylococcal Infections. The therapeutic effect of sulphanilamide, "disulphanilamide," the dimethyl derivative of disulphanilamide, in staphylococcal infections in mice, is shown in Table XII.

Although the final results of treatment with these three compounds are not striking, it is perhaps significant that while 82 per cent of the untreated control mice had died before the end of the 10 day treatment period, 76 per cent of the "disulphanilamide" mice, 64 per cent of the sulphanilamide and 48 per cent of the dimethyl derivative of disulphanilamide series were alive at this time.

Domagk's (21) results from the peroral treatment of staphylococcal infections in mice with the dimethyl derivative of "disulphanilamide" ("Uliron") were considerably better than those we are reporting. Domagk found that an infection which caused the death of 91.7 per cent of the control mice, would, if treated with Uliron, show a death rate of 13 to 31 per cent depending upon the size of the mice. However, his mice were infected by the intraperitoneal route and in all probability their deaths were due to a toxemia rather than to a generalized infection. Moreover, they were observed for only six days. Domagk also reported that 8 out of 14 mice treated with various doses of "disulphanilamide" survived a similar staphylococcal infection.

Our results are similar to those reported recently by Mellon,

TABLE XII

The therapeutic effect of prolonged treatment with sulphanilamide and certain similar compounds upon experimental staphylococcal infections in mice

COMPOUND	NUMBER OF MICE	DOSAGE*	DEATHS, DAYS																											SURVIVORS	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30‡	No.	%
Sulphanilamide	50	10.0 mg. BID 5 QD 5	1	2	1	1	2	2	2	5	1	1	1	3	1	2	1		2			1	1			1			2				17	34
Disulphanilamide	50	10.0 mg. BID 5 QD 5		2	1	2		2	5					2		3	2		2	2	2		2								1		22	44
Dimethyl derivative of Disulphanilamide	50	10.0 mg. BID 5 QD 5	2	3	3	3	3	2	5	2	2	1				1	1		1			1					2			2		1	15	30
Controls	50		9	3	3	3	7	11	5				2				2																5	10

* See footnote table IX.

‡ Several mice died after the 30 day period.

Shinn and McBroom (22) for staphylococcus aureus infections in mice treated with the same compounds. Any difference between the two series can be accounted for by differences in the virulence of the cultures and in the duration of therapy.

Recently, two other compounds, 4:4′ diamino diphenyl sulphone and di(acetylamino) diphenyl sulphone have been tested against staphylococcal infections in a small series of mice. In this experiment the 10 controls were all dead in the 10-day treatment period, while 10 out of 15 mice in each of the two treated groups were dead.

DISCUSSION

A consideration of the data presented in this report brings out the following points of interest. In the first place, it seems that the conclusions arrived at by certain observers (14, 15, 16, 18) concerning the acute toxicity for mice of insoluble chemotherapeutic compounds must be revised. It is impressive to record that 20, 30 or 40 grams per kilogram of mouse body weight of a given compound can be given to mice without toxic effects, but if evidence is available that only one gram may be absorbed, then such figures have little significance. This is well illustrated by "disulphanilamide." Rosenthal (14) reported that doses of 8 grams per kilo were non-toxic in mice and Barlow (16) stated that mice receiving up to 40 grams per kilogram showed no toxic effects. Actually it appears from the blood levels of "disulphanilamide" which we have noted in mice following various doses of this compound that there is no increase in the absorption of the compound after doses of 1 gram per kilogram are passed. This finding is to be contrasted with our observations upon the blood levels obtained following increasing doses of sulphanilamide in mice. In this instance there is evidence of continued absorption until toxic levels are reached.

It may be said that blood levels of a given compound are not indicative of true absorption when data regarding the renal clearance are lacking. However, with "disulphanilamide," Marshall (23) has shown that the renal clearance is four times that of sulphanilamide. Even after taking this observation into consideration it is still obvious that the absorption of "disulphanilamide" is at a definitely low level. It is our belief that the same is true of the dimethyl

derivative of "disulphanilamide" and of para benzyl amino benzene sulphonamide.

The blood levels following increasing doses of diamino diphenyl sulphone are of interest in that the maximum blood levels are obtained at a dosage which is lethally toxic for the mice. In this respect the diamino diphenyl sulphone resembles sulphanilamide.

We (24) have reported that strong reducing agents will reduce "Prontosil Solution" to sulphanilamide *in vitro*. Fuller (25) has shown that a certain proportion of injected "Prontosil Solution" or ingested "Prontosil" may be recovered from the urine of human beings as free sulphanilamide, and Rosenthal states (14) that in rabbits "85 to 95 per cent" of the injected "Prontosil Solution" was recovered from the urine within 5 hours after intramuscular or intravenous injection. Our experience shows that, following the injection of "Prontosil Solution" into mice, there is an increasing amount of free sulphanilamide in the blood up to the fourth hour after injection. When the blood level of free sulphanilamide at 4 hours in mice injected with 6 mgms. of sulphanilamide was compared with the levels obtained following the injection of a molar equivalent of "Prontosil Soluble" (20.5 mgms.) it was found that the levels were similar. It was also of interest to note that the administration of 1, 2, 3 or 4 grams per kilogram of "Prontosil Soluble" *per os* to mice did not result in markedly different sulphanilamide blood levels four hours after the administration of the drug. This finding was unexpected because of the excellent solubility (5 per cent) of the compound. It may be due to the fact that in the mouse the possible reducing systems active in reducing "Prontosil Soluble" to sulphanilamide can take care of only a given amount of the compound and that, when such an amount is exceeded, no increase in reduction can be expected.

It is obvious from our studies that a proper evaluation of the toxicity of a chemotherapeutic compound cannot be made unless data regarding the absorption and excretion of the compound are available. Familiarity with the absorption of a compound is of great value in determing the requisite size and frequency of dosage in the treatment of experimental infections in mice.

One of the greatest problems which confronts those who are in-

terested in the chemotherapy of bacterial infections is that of the comparative therapeutic efficiency of various compounds. Clearly, it would take years to accumulate enough clinical data regarding any one compound to be of statistical significance. Because of this, it is necessary to resort to experimental infections in laboratory animals. Fortunately, experimental infections in mice offer a fairly satisfactory method for the testing of new compounds *if statistically significant numbers of the animals are used.*

During the past year we have been interested in testing the comparative therapeutic values of sulphanilamide and "Prontosil Solution." If our thesis that "Prontosil Solution" acts by being broken down to sulphanilamide in the animal body is correct (6), then the molar equivalent of 6 mgms. of sulphanilamide in terms of "Prontosil Soluble" should give essentially the same survival rate as the sulphanilamide. When put to test, this is exactly what happened. In this respect our observations differ from those of Rosenthal (14), but his results are reported upon the basis of only ten mice in each group.

The observation that molar equivalents of sulphanilamide and "Prontosil Soluble" gave similar therapeutic results in experimental streptococcal infections is in harmony with our previous observations upon the breakdown of the compound *in vitro* and upon the blood levels of free sulphanilamide obtained in mice following the administration of the compound. It seems, therefore, that in so far as the mouse is concerned, the action of "Prontosil Soluble" can be attributed to its breakdown to sulphanilamide.

When an attempt is made to compare results obtained here in the treatment of experimental infections with chemotherapeutic compounds, with those results obtained by other observers, certain obstacles immediately appear. It is well known that the constituents of the media, the age of the culture and the methods of dilution have definite bearing upon the virulence of streptococci for mice. Then, too, the general health of the mice under test, the methods of administration of the chemotherapeutic agents, the spacing of the doses of the compound and the length of time the mice are observed all enter into the problem whether observer A's results can be compared with those of observer B.

Nevertheless, we propose to discuss the results which we have

obtained in comparison with those reported by other workers. In general the results which we have noted regarding the chemotherapeutic efficiency of sulphanilamide in experimental streptococcal infections are similar to those of other observers. Our observations regarding the efficiency of "disulphanilamide" are in harmony with those of Rosenthal (14) when he used this compound orally in the treatment of streptococcal infections in mice. However, in computing the therapeutic index for "disulphanilamide," Rosenthal based his therapeutic results upon mice treated by parenteral injections of "disulphanilamide" suspended in olive oil (a completely impractical method) and arrives at the conclusion that "disulphanilamide" has a "therapeutic index at least five times as favorable as sulphanilamide." A single protocol in which 10 mice were used in testing the therapeutic values of sulphanilamide and "disulphanilamide" in streptococcal infections is recorded in Rosenthal's paper.

Barlow (16) has recently reported that "disulphanilamide" ("Disulon"), due to its lower toxicity, better tolerance and the greater protective efficiency of unit dosages in the presence of infections, has a therapeutic margin of safety quite superior to that of "Prontylin."

It is our conclusion, after taking into consideration the blood levels obtained after its administration, that "disulphanilamide" may be slightly more active than "sulphanilamide" in experimental streptococcal infections. But, because of its poor absorption and a general lack of knowledge concerning the acute toxicity of this compound, we believe further experimental work is necessary before it can safely be introduced into the clinic.

The dimethyl derivative of "disulphanilamide" ("Uliron") has been reported by Domagk (21) to be an effective chemotherapeutic agent in experimental streptococcal infections upon the basis of tests on 20 mice. Also, in this instance, the compound used was the sodium salt and not the insoluble dimethyl derivative itself. Our experience with the insoluble compound in the treatment of streptococcal infections in mice leads us to believe that it is *definitely inferior* to sulphanilamide and "disulphanilamide." Furthermore, its acute toxicity by the oral route cannot be determined because of the low rate of absorption of the compound.

Para benzyl amino benzene sulphonamide ("Septazine," "Setazine")

has been reported by Halpern and Mayer (15) and by Mayer (18) to be as active as sulfamidochrysöidine and sulphanilamide in the treatment of experimental streptococcal infections in mice. Whitby (26) states that, "the oral preparations, p—benzyl amino benzene-sulphonamide and p—aminobenzene sulphonamide, are equally effective in experimental streptococcal infections. The former is very much less toxic than the latter." Our experience has been that the benzyl derivative is the least effective of the compounds described in this communication. This lack of effectiveness, coupled with the fact that nothing is really known concerning the absorption or excretion of this compound (no practical method of determining this agent is available) makes us feel that this almost insoluble compound is definitely inferior to sulphanilamide for use in hemolytic streptococcal infections in man.

As has been previously noted by Buttle *et al.* (13) and by Fourneau and his associates (27), diamino diphenyl sulphone is the most efficient chemotherapeutic agent yet described for the treatment of experimental streptococcal infections. Unfortunately, it is a highly toxic compound and for this reason it has not so far been suggested for clinical use.

The acetylated derivative of the diamino sulphone is relatively non-toxic for mice. Blood levels obtained in mice following oral doses of the di(acetylamino) compound seem to indicate that its absorption is somewhat superior to that of the diamino sulphone. Actually, however, we are inclined to the belief that its lack of acute toxicity is, as is the case with other practically insoluble compounds, the result of a lack of absorption. The results obtained in the treatment of experimental infections in mice are of some interest. Fourneau and his collaborators (28) have reported the di(acetylamino) derivative to be as active in streptococcal infections in mice as the non-acetylated compound. Unfortunately, however, in their published report, only small numbers of mice are discussed and the data presented are not adequate. We have found that in mice treated immediately after intraperitoneal inoculation of 100–1,000 M.L.D. of a virulent strain of the hemolytic streptococcus (C2O3) the survival rate at the end of 14 days was definitely better than was obtained under similar conditions with sulphanilamide. If, however, the streptococcal infection

was permitted to develop before treatment was instituted, then di-(acetylamino) diphenyl sulphone gave results which were somewhat inferior to those obtained with sulphanilamide. This paradoxical finding cannot be explained at the present time. The activity of this compound against experimental streptococcal infections is, in our opinion, great enough to warrant a careful clinical study of its effects in streptococcal infections in human beings.

Diamino diphenyl sulphone was the sole compound under investigation which showed a real degree of therapeutic activity in experimental pneumococcal infections in mice. Buttle *et al.* (13) had previously noted this effect as had Fourneau, the Tréfouëls, Nitti and Bovet (28). The di(acetylamino) derivative has been reported by the Tréfouëls, Nitti and Bovet (29) as being active against pneumococcal infections in mice. Our data indicate that, while this compound has some degree of activity in experimental pneumococcal infections in mice, its effect is not greater than that of sulphanilamide.

It has been shown by Buttle (4), Proom (30), Rosenthal (31) and Brown (8) that sulphanilamide is an effective chemotherapeutic agent in the treatment of experimental meningococcal infections in mice. Rosenthal (14) has reported that a single parenteral treatment with "disulphanilamide" suspended in oil would save 90 per cent of mice previously infected with meningococci and that in meningococcal infections in mice, "disulphanilamide" injected subcutaneously was superior to para benzyl amino benzene sulphonamide or sulphanilamide as a therapeutic agent. Whitby (26) reported that benzylamino benzene sulphonamide was without effect in meningococcal infections. Our experience showed that sulphanilamide, "disulphanilamide," diamino diphenyl sulphone and di(acetylamino) diphenyl sulphone were all about equal in activity against experimental meningococcal infections in mice, while the dimethyl derivative and para benzyl amino benzene sulphonamide were definitely less active when these compounds were administered by the oral route.

Domagk (21) has reported that the dimethyl derivative of "disulphanilamide" ("Uliron") given in a watery suspension, or as the sodium salt in solution, had a definite curative effect in mice infected by the peritoneal route with staphylococci. Mellon and his associates (22) failed to confirm Domagk's observations in respect

to the effect of the dimethyl derivative, but reported that "disulphanilamide" had an "apparent therapeutic effect over a limited time period" in experimental staphylococcal infections in mice. Mellon infected his experimental mice by intravenous injections of staphylococci.

Our results in the treatment of mice infected by the intravenous route with staphylococci were essentially in harmony with those obtained by Mellon and his coworkers (22), although our data showed a better therapeutic effect from the dimethyl derivative than did those of Mellon. It is our belief that these observations indicate that sulphanilamide, "disulphanilamide" and its dimethyl derivative are of little value in invasive tissue infections in the mouse due to staphylococci, and that Domagk's observations in this respect indicate that he was dealing with a non-invasive type of staphylococcal infection.

CONCLUSIONS

I. Sulphanilamide is a highly effective compound in the treatment of experimental streptococcal infections in mice. 4:4′ diamino diphenyl sulphone is more active in experimental streptococcal infections than is sulphanilamide, but is also highly toxic for mice. Di(acetylamino) diphenyl sulphone shows a degree of activity in experimental streptococcal infections in mice which warrants a careful clinical trial of this compound in hemolytic streptococcal infections in man. "Disulphanilamide," its dimethyl derivative, and para benzyl amino benzene sulphonamide show a moderate degree of activity in experimental streptococcal infections in mice.

II. Further experimental evidence indicates that the activity of "Prontosil Solution" is dependent on its reduction to sulphanilamide *in vivo*.

III. 4:4′ diamino diphenyl sulphone has a real degree of therapeutic activity in experimental types I and II pneumococcal infections. Its toxicity, in our opinion, precludes its use in human beings. Sulphanilamide, "disulphanilamide," the dimethyl derivative of "disulphanilamide," di(acetylamino) diphenyl sulphone and para benzyl amino benzene sulphonamide showed little or no therapeutic activity in experimental pneumococcal infections in mice.

IV. In experimental meningococcal infections in mice, sulphanilamide, "disulphanilamide," diamino diphenyl sulphone and di-(acetylamino) diphenyl sulphone all showed about the same degree of therapeutic activity. The dimethyl derivative of "disulphanilamide" and benzyl amino benzene sulphonamide were definitely less active as therapeutic agents.

V. Sulphanilamide, "disulphanilamide" and its dimethyl derivative showed low degrees of activity in experimental staphylococcal infections in mice.

VI. The reported lack of acute toxicity in mice given large doses of "disulphanilamide" is dependent upon the fact that the drug is not well absorbed. This is probably also true for other almost insoluble compounds.

VII. The experimental data recorded in this paper indicate that sulphanilamide is from all points of view the most suitable compound for the treatment of hemolytic streptococcal and meningococcal infections in human beings. It is of doubtful value in pneumococcal and staphylococcal infections.

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ON THE OCCURRENCE OF PHARYNGEAL INFECTIONS IN EXOPHTHALMIC GOITER*

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To establish the etiology of a disease of human beings, the investigator should discover a common denominator beyond reasonable doubt. If a denominator of a pathogenic nature can be found among patients with exophthalmic goiter, it should be given more weight than can be attributed to other factors which are rarely or never found in action at the bedside, no matter how definite their influence may be. Since exophthalmic goiter is a human disease, reproduced rarely if ever in animals in typical form, it behooves the practising physician to search for the factor that is most characteristic in the background of the patients who consult him.

There seems to be no doubt that various endocrine, dietary, and nervous influences act on the thyroid gland in a fundamental manner. The physician, however, finds it difficult to apply the knowledge of these factors, gained through experimental methods, to his patients, or to determine that any of these agents are actually at work in the patient with exophthalmic goiter. An important pathologic feature of the disease is the lymphoid hyperplasia of the pharynx and of the cervical glands—frequently noted clinically and emphasized more than thirty years ago by MacCallum (13). Although it has become customary to consider such changes as secondary to the goiter, this concept is far from convincing.

It is clear from a survey of the literature, too abundant for full consideration here, that many laryngologists and practising physicians have held to the infectious theory of exophthalmic goiter. The reports have dealt largely with tonsillitis and rheumatic fever as the most likely infectious agents.

* Read before the Association of American Physicians, May, 1937.

Personal observation to the effect that a patient with exophthalmic goiter is often a patient with an unusual degree of pharyngeal infection has led the writer to consult the records of certain cases with which he was familiar; it also seemed advisable to extend such casual observations by an investigation of the possible importance of such infections in quite a number of cases of exophthalmic goiter. The following cases illustrate the question under consideration:

A. B., fifty-three year old male. He had been submitted to a thyroidectomy for exophthalmic goiter by Dr. J. M. T. Finney four years before I saw him in 1926. He had been symptomatically well following this operation. Shortly before I saw him he had had another severe sore throat, and, when examined, had a recurrence of exophthalmic goiter with B.M.R. plus 52%, evidences of chronic tonsillitis, and attacks of auricular fibrillation. At my suggestion tonsillectomy was performed, and infected tonsils were removed. The exophthalmic goiter, however, persisted, and about a month after tonsillectomy a second thyroidectomy was done. He wrote me, March 7, 1934—eight years after his tonsillectomy and second thyroidectomy—that he was perfectly well.

K. T., a fifty-year old white woman, had undergone a thyroidectomy eighteen years prior to admission. During her life she had had several attacks of tonsillitis. After her first thyroidectomy she had remained well in all respects until shortly before admission. She then suffered from an attack of influenza complicated by sore throat. Her tonsils were involved in this infection. Shortly after this attack she developed enlargement of the thyroid gland, lost weight and had other typical symptoms of exophthalmic goiter. After her return home, I found her tonsils ragged but small, the left cryptic, containing some débris. B.M.R. plus 41%. A laryngologist reported the tonsils large, adherent, chronically infected. There was a slight mitral insufficiency. Tonsillectomy was done and cultures showed (1) Beta hemolytic streptococci; (2), Alpha hemolytic streptococci; and (3) staphylococci. There was no substantial change in the thyroid condition following the tonsillectomy, and five weeks later thyroidectomy was performed. Section of gland: exophthalmic goiter. Six years later the patient was perfectly well.

E. N., fifteen-year old white girl, admitted for thyroid trouble and tonsillitis. She had had a moderate number of sore throats. During the two years before admission she had had four attacks of tonsillitis. For two months prior to examination she had had a sore throat almost constantly. This was followed by typical exophthalmic goiter, with B.M.R. plus 24%. The tonsils were tremendously enlarged, some exudate on the right, with markedly enlarged tender glands at angles of the jaw. Throat culture showed Beta hemolytic streptococci moderately predominant, also Alpha hemolytic streptococci. Because of the coincidence of tonsillitis and goiter, tonsillectomy and adenoidectomy were performed. Two weeks later the thyroid seemed smaller and firmer and the

B.M.R. was plus 11%. One month after tonsillectomy the B.M.R. was plus 1%, resting pulse, 81–86. The exophthalmos was still present, the patient had lost some weight, but there was less tremor. This patient received no iodine. *Note made November 13, 1934:* On this patient's admission, October–November, 1933, subsequent throat cultures showed in addition to the above two types of streptococci: hemophilus hemolyticus, staphylococcus aureus, neisseria, non-hemolytic staphylococcus, hemophilus influenzae. After tonsillectomy, from cut surface of tonsils, Alpha streptococcus predominated, also minute Beta streptococcus, hemophilus influenzae, also staphylococcus aureus. As noted, B.M.R. plus 1%, January 5, 1934. Pulse 81 to 86. On April 4, 1934, pulse 116. Return of thyroid symptoms. Admitted to hospital with diffusely enlarged thyroid, exophthalmos, tremor, B.M.R. plus 54%. Under Lugol's and rest B.M.R. fell to plus 14%. Double partial lobectomy April 24, 1934. *Pathologic diagnosis:* Diffuse goiter with marked epithelial hyperplasia (high columnar cells, several layers in thickness). Papillary infolding and lacy border of the colloid and solid growth of cellular tissue quite conspicuous. Connective-tissue trabeculae somewhat increased. Three years later this patient was quite well.

G. J., a twenty-nine year old negress, had had "diphtheria" at fifteen. After this she had had tonsillitis every winter. For seven years she had had symptoms of mild exophthalmic goiter. On admission to the Johns Hopkins Hospital she had acute tonsillitis, due to Beta hemolytic streptococcus, cervical adenitis, and well marked exophthalmic goiter (B.M.R. plus 69%). The Wassermann reaction was positive. Thyroidectomy was performed. Tonsillectomy will be done later.

E. D., a thirty-four year old white woman, gave the history of the usual childhood disorders. On admission to the hospital she dated her more serious symptoms to an attack of scarlet fever in 1929, when she was in bed for six weeks. During the ensuing two years she developed definite symptoms, including gradual swelling of the thyroid gland, which had required thyroidectomy in 1931. In 1936 she had a recurrence of the thyroid symptoms, with a metabolic rate of plus 74%. The tonsils were somewhat glazed and adherent, the pharynx injected, and there were palpable glands at the angles of the jaw. Thyroidectomy was performed again.

Mr. L., a thirty-four year old white man, whose mother had undergone a thyroid operation, had suffered from frequent tonsillitis all his life. At times this was complicated by cervical adenitis. There was no other serious infection. Two years before consulting me he had an unusually severe sore throat, after which a tonsillectomy was performed. A few months later, however, he began to have symptoms of exophthalmic goiter, which were present in typical form when I saw him. Though the metabolic rate was only plus 12.5%, the resting pulse was 100 and all the classical symptoms were present.

H. I., a very intelligent daughter of a physician, unmarried, aged thirty-five, had had the usual childhood diseases. At the age of five she had chorea. A year later her tonsils were removed. She was well and at work in New York when she

developed a severe sore throat. Cultures on several occasions showed a hemolytic streptococcus which finally disappeared from the flora of the throat. During the following few months she lost twenty pounds in weight, was nervous and tremulous. Six months after the throat infection, when I saw her, she had typical exophthalmic goiter, with a metabolic rate of plus 54%. The pharynx had become normal in appearance. Eighteen months after double partial lobectomy she was well.

C. and E., twins: Dr. W. F. Rienhoff, Jr. gave me the opportunity of examining two twin white girls, aged 17, one of whom (E.) had exophthalmic goiter. They were of very similar appearance, though it was not known whether they were "identical" twins or not. They had had identical childhood diseases, including diphtheria at the age of three. Between eight and thirteen years of age they each had rather frequent sore throats and tonsillectomy was performed on each at the age of thirteen. The only deviation of the history of one from that of the other lay in the fact that E. developed cervical adenitis shortly before her tonsillectomy, while C., the healthy sister, failed to do so. E. developed exophthalmic goiter for which a double partial lobectomy was done at the age of seventeen. Her metabolic rate before operation was +37.5%. C. was perfectly well.

W. T., white, male, aged 28, on admission to the Johns Hopkins Hospital gave the following history. He had had rheumatic fever in 1921 and had been subject to tonsillitis before his entrance into the hospital in 1932 with exophthalmic goiter. His metabolic rate was about 25% plus. He showed enormous, infected tonsils and during treatment preparatory to operation he had an acute tonsillitis; throat culture showed the temporary presence of Beta hemolytic streptococci in pure culture. He also showed cervical adenitis and a mitral insufficiency. Double partial lobectomy was performed. While convalescent on the surgical ward he developed an active rheumatic fever complicated by acute pericarditis. After his recovery, tonsillectomy was performed. Four years later he was well save for certain mental and domestic problems. The metabolic rate was minus 1%. He still had signs of mitral insufficiency.

This brief series of cases, which could be amplified almost indefinitely, serves to indicate the possible etiologic, or possible accompanying rôle of infections in exophthalmic goiter. All these patients had had attacks of tonsillitis, one had had chorea, two had had mitral insufficiency and one frank rheumatic fever. Only one had a positive Wassermann reaction, and there was no clue that any infection except those in the pharynx and the "rheumatic infection" in general was concerned in their condition. In 3 cases in which throat cultures were made and in 1 case in which cultures from removed tonsils were examined, a hemolytic streptococcus was found in pure culture or in predominant numbers. In 3 cases a previous thyroidectomy had

TABLE A

Cases of exophthalmic goiter following infection

NAME	AGE	SEX	HISTORY	NATURE OF INFECTION	RESULT
A. B.	53	M	Thyroidectomy 1922. Recurrence of exophthalmic goiter 1926, after U. R. I.	"Severe sore throat." Removed tonsils contained pus	Well 8 yrs. after second thyroidectomy and tonsillectomy
K. T.	50	F	Frequent tonsillitis in early life. Thyroidectomy 1914. Recurrence 1932	"Influenza with involvement of tonsils." T. & A. culture: B. h. strept. *et al.*	Well 6 yrs. after T. & A. and 2nd thyroidectomy
E. N.	15	F	Moderate no. sore throats 4 X tonsillitis in 2 yrs. Constant tonsillitis 2 mos.	Chronic tonsillitis. Culture: B. h. strept. *et al.* Adenitis	T. & A. unsuccessful. Well 3 yrs. after thyroidectomy
G. J.*	29	F	Tonsillitis every winter, following "diphtheria" at 15	Acute tonsillitis, due to B. h. strept. Adenitis. W. R. + Lymph. ven.	Thyroidectomy
E. D.	34	F	Scarlet fever 1929. Tonsillitis & thyroid symptoms 1929–31. Thyroidectomy 1931. Recurrence 1936	Scarlet fever (history). Adenitis 1936	Thyroidectomy
L.	34	M	Tonsillitis always. Adenitis. Severe tonsillitis & T. & A. 1935. Exophthalmic goiter 1935–37	Tonsillitis. Adenitis	
H. I.	35	F	Tonsillectomy as child. Pharyngitis 1935 (Mar.). Exophthalmic goiter, Sept. 1935	H. strept.	Well 2 yrs. after thyroidectomy
S. twins	17	F	Parallel child. dis. Tonsillitis 8—13 yrs. T. & A. at 13	E.—cerv. ad. & exoph. goiter. C.—No cerv. ad. & no exoph. goiter	E. Thyroidectomy
W. T.	28	M	Tonsillitis. Rheum. f. 1921 & 1932, after lobectomy. T. & A. 1932	B. h. strept. with tonsillitis. Rheumatic fever	Well, with mitral insufficiency

* Colored.

been performed and the tonsils had been allowed to remain; in two of these recurrence of thyroid symptoms followed acute tonsillitis, and in a third case recurrence was associated with an acute exacerbation of heavily infected tonsils. One case followed scarlet fever, another a streptococcus sore throat. The diagnosis of exophthalmic goiter was verified by the pathologist in every case in which operation was performed. There was no indication of thyroiditis.

The infectious theory for the etiology of exophthalmic goiter is supported by many previous reports. The name of McCarrison (14) is prominent in this connection, but this observer, impressed perhaps by his animal experimentation, preferred to believe that infected material from tonsils and gums acted to predispose certain individuals to exophthalmic goiter after being ingested and reaching the thyroid through the blood stream. Likewise, Thompson (18), who was much impressed by the rôle of tonsillitis in causing exacerbations of the disease, was deterred from assuming that the thyroid could be influenced directly by tonsillar infection because of the apparent lack of direct blood-vascular or lymphatic connection between throat and thyroid. Keilty (11), after an extensive study of tonsils and thyroid gland, both as regards bacteriology and pathology, concluded that, in a majority of cases, the disorder is an inflammatory reaction due to the response to bacterial stimuli. De Quervain (5) recognized a non-pyogenic response in the thyroid to rheumatic and pharyngeal infections. Other clinical reports of cases of exophthalmic goiter in individuals with diseased tonsils are so numerous that it is impossible to do justice to them here (1, 2, 3, 7, 8, 9, 12, 15, 17).

Solution by the experimental method of the immediate cause of this disease is difficult or impossible owing to its peculiar limitation to human beings. However, the early observations of Halsted (10), that infection in the operative wound leading to the dog's thyroid may cause subsequent enlargement of the gland, supported his similar results from infections set up in the peritoneal cavity. Cole and Womack (4), producing focal infections with streptococci and staphylococci, were able to demonstrate changes in the thyroid of the dog that were scarcely to be distinguished from those of human exophthalmic goiter. However, in order to substantiate or disprove the common observation at the bedside that exophthalmic goiter follows

tonsillitis, one would have to set up a chronic infection, probably streptococcal, in the tonsils of animals and cause these infections to undergo repeated acute exacerbations.

Derick and Fulton (6) have investigated various diseases as regards the sensitiveness of the skin of patients to the injection of a nucleoprotein fraction derived from hemolytic and green streptococci. Patients with rheumatic fever or chorea showed the largest incidence of positive reactions; in exophthalmic goiter, positive tests were obtained in 75 per cent of the cases, while a similar proportion obtained in tonsillitis and sinusitis. Normal individuals reacted with 54.9 per cent positive results.

While many clinical reports have supported the concept that the immediate or precipitating factor in causing exophthalmic goiter may be an infection in the upper respiratory tract, the material on the whole is somewhat inconclusive for two reasons: (1) there has been no adequate attempt to determine how common such infections are in diseases in which the infection is known not to play a part; and (2) there has been a rather wide belief that no intimate lymphatic connection exists between tonsils and thyroid.

I have attempted to throw some light on the first of these problems by consulting the files of the Johns Hopkins Hospital, from which 200 cases of exophthalmic goiter were analysed.[1]

Only histories of patients admitted to the Medical Service were used, since the routine history and physical examination are somewhat more elaborate than is the case on other Services. In the examination of the throat the diagnosis of a laryngologist was selected wherever available, otherwise that of the medical examiner. The first item had to do with the history. Under this heading an attempt was made to determine whether the patient had suffered from an excessive number of attacks of tonsillitis or sore throat, or from sore throats of excessive severity. It is not possible always to do this accurately, but a similar procedure was carried out in the "control" cases, as will be shown in the tables. There is no special comment

[1] To eliminate personal bias in the analysis of histories, I left this work so far as possible in the hands of Miss Mary Stoll; she was not told the nature of the thesis that was being tested.

to be made on the history of tonsillectomy, though, of course, the tonsils are not always removed completely. I think it is safe to say that tonsillectomy was done in these cases on objective grounds: that is to say, I do not think tonsillectomy has been done in Baltimore in many cases merely because of the existence of exophthalmic goiter. Nor do we make the diagnosis of chronic tonsillitis with perfect accuracy; however, the same criteria were used in the case of exophthalmic goiter as in the "control" groups. Excessive scarring, glazing or adhesions are considered evidence of past infection, while pus which can be expressed, obvious white inclusions and enlarged glands at the angles of the jaw are held to be important. The last analysis consists of those patients who gave a history of no more than the usual number of ordinary sore throats, had never been submitted to a tonsillectomy, and had no evidence of definite tonsil infection when examined.

In selecting control groups many considerations arose. The chronic arthritis patients and all those showing the complications of rheumatic fever, such as valvular heart disease, must be eliminated, as they are recognized as frequently having associated throat infections. I selected 200 patients with tuberculosis and 200 with cancer. These groups are not ideal. For one thing, tuberculous tonsils are by no means uncommon, as sections from routine tonsillectomies at the Johns Hopkins Hospital have shown. On the other hand, some have had the impression that cancer patients have had on the whole less than their share of respiratory tract infections, though it is not easy to obtain the facts on this subject. After some thought I selected a third group of control cases—syphilitic patients. Such individuals should represent a fair cross-section of the population, so far as respiratory infections are concerned, though they would be apt to come from the lower strata of society and to use poorer personal hygiene.

COMMENT ON TABLES

From the statistical point of view, Dr. Margaret Merrell, of the Johns Hopkins School of Hygiene and Public Health, has been kind enough to advise me that the likelihood of a difference as great as that found in the comparison of 200 cases of exophthalmic goiter with 200 cases of syphilis being due to pure sampling is about four

in ten thousand. When the comparison is made between two hundred cases of exophthalmic goiter and six hundred control cases, the likelihood of this being due to error in sampling is reduced, to all intents and purposes, to zero. This being the case, these data must be examined for various other influencing factors.

It is clear that common sense considerations may be involved in such a statistical study—especially such factors as color, age and sex. The group of syphilitic cases had an average age of 36.08 years as

TABLE 1

Exophthalmic goiter (200 cases) and 600 controls (Tbc., cancer, syphilis)

	POSITIVE HISTORY	T. AND A.	PRESENT INFECTION	RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE	ENTIRELY NORMAL
Exophthalmic goiter	67.5	28.0	48.0	14.0	17.5
Controls	48.3	17.0	11.8	10.3	34.5

TABLE 2

Throat infections in various conditions

Percentage

	POSITIVE HISTORY	T. AND A.	PRESENT INFECTION	RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE	ENTIRELY NORMAL
Exophthalmic goiter	67.5	28.0	48.0	14.0	17.5
Syphilis	53.5	18.0	13.5	10.0	32.5
Tuberculosis	51.0	24.5	14.5	8.0	26.5
Cancer	40.5	8.5	7.5	13.0	44.5

compared with an average age of 35.68 years in the exophthalmic goiter cases. The syphilitic cases were selected so as to contain the same number of white and black patients as were found in the unselected exophthalmic goiter cases.

In comparing the exophthalmic goiter and syphilis groups there remains only the question of sex. About two-thirds of our patients with exophthalmic goiter were women. Could the high incidence of tonsil infection in this group be a characteristic of the female sex rather than of exophthamic goiter? Curiously enough, when these cases were separated into female and male groups, it was found that

the women had given a higher proportion of "positive throat histories" than the men (74 per cent F: 53 per cent M): objective examinations, however, showed that tonsillectomy was done in 29 per cent of females as against 27 per cent of males, while a higher proportion of males had infected tonsils when admitted for exophthalmic goiter (52 per cent M: 46 per cent F). Among 200 male and 200 female patients with syphilis, tuberculosis and cancer the comparison between the sexes is as follows:

Thus, it is clear that the influence of sex on liability to tonsillectomy and on the objective occurrence of diseased tonsils is negligible. However, both in the exophthalmic goiter and in the control series, women give a slightly higher proportion of histories of excessive throat infections in the past. When the figure showing the incidence of such infections as "positive history" in the analysis of exophthalmic goiter

TABLE 3

	POSITIVE HISTORY	T. AND A.	PRESENT INFECTION
	per cent	*per cent*	*per cent*
200 males	49.5	20	11
200 females	56.5	21	10.5

is corrected for the sex influence a figure of about 61 per cent is obtained. This figure remains higher than that of any in the "control" groups.

With such a correction for sex, a fair comparison is possible between the cases of exophthalmic goiter and syphilis. When all 600 "control" cases are merged, the average age of the "control" cases becomes about five years higher than that of the exophthalmic goiter cases, owing to the inclusion of cancer patients. The error due to sampling, however, is reduced, to all intents and purposes, to nil. Age might influence the figures in that certain patients might forget earlier infections, and evidences of such infections might tend to disappear; on the other hand, the longer a person lives the greater the stretch of time over which infections may occur and tonsillectomy may be done.

Thus, the patients with exophthalmic goiter surpass the "control" groups of patients in every department—history of infection, incidence

of tonsillectomy and evidence of infection on examination. How do the exophthalmic goiter cases compare with a group in which throat infections are known to be important? To decide that question we selected 150 cases of rheumatic fever from the files of the Johns Hopkins Hospital.

It is clear that a close similarity exists between the two groups. If the figures are divided into those based on history the difference is only 3 per cent. If the figures from objective examinations (tonsillectomy and direct inspection of the throat) are merged, rheumatic fever patients show an incidence of 36 + 39.3 = 75.3 per cent; the exophthalmic goiter cases show an incidence of 28 + 48 = 76 per cent.

The great divergence of exophthalmic goiter cases from the controls is in the incidence of diseased tonsils found on admission to hospital.

TABLE 4

Rheumatic fever (150 cases) and exophthalmic goiter (200 cases)

Percentage

	POSITIVE HISTORY	T. AND A.	PRESENT INFECTION	PREVIOUS RHEUMATIC FEVER AND RHEUMATIC HEART DISEASE	ENTIRELY NORMAL
Rheumatic fever	70.6	36.0	39.3	38.6	11.3
Exophthalmic goiter	67.5	28.0	48.0	14.0	17.5

The question naturally arises whether such throat infections are secondary to exophthalmic goiter, as has been suggested. In the first place, the patients' histories indicated an incidence of previous tonsillitis in exophthalmic goiter cases almost as high as in those of rheumatic fever and substantially higher than in the controls. The cases of exophthalmic goiter in which tonsillectomy was done were analysed, with results shown in Table 5, tonsillectomy being used as an index to the degree of throat infection. Of the 56 cases, 40 were performed before the onset of symptoms of exophthalmic goiter, and 15 after the onset. In one case the date of operation was not given. In two of the 15 late tonsillectomies, no history of previous throat infection was noted: it is, therefore, possible to assume that the tonsil infection might have followed the onset of exophthalmic goiter. In 8 of these 15 cases there was a clear history of tonsillitis antedating

the onset of thyroid symptoms. Of the remaining 5, a history of excessive sore throats was clear in 3, equivocal in 2 others. I have been unable to convince myself that the throat infections in any substantial number of cases could be attributed to primary occurrence of exophthalmic goiter.

If throat infections are concerned in thyroid disturbances, it seems to-day unnecessary to follow McCarrison's (14) hypothesis that the toxic agent is ingested and affects the thyroid through the blood stream. Troell (19) recognized the relation that exists between the thyroid gland, the cervical lymphatics and the tonsils. He removed neighboring lymph glands in several thyroid operations: in all, the picture was one of acute inflammation. He found round cell infiltration in 95 per cent of thyroid glands removed for exophthalmic goiter and considered this finding an indication of infection but credited Holmgren with first advocating this concept.

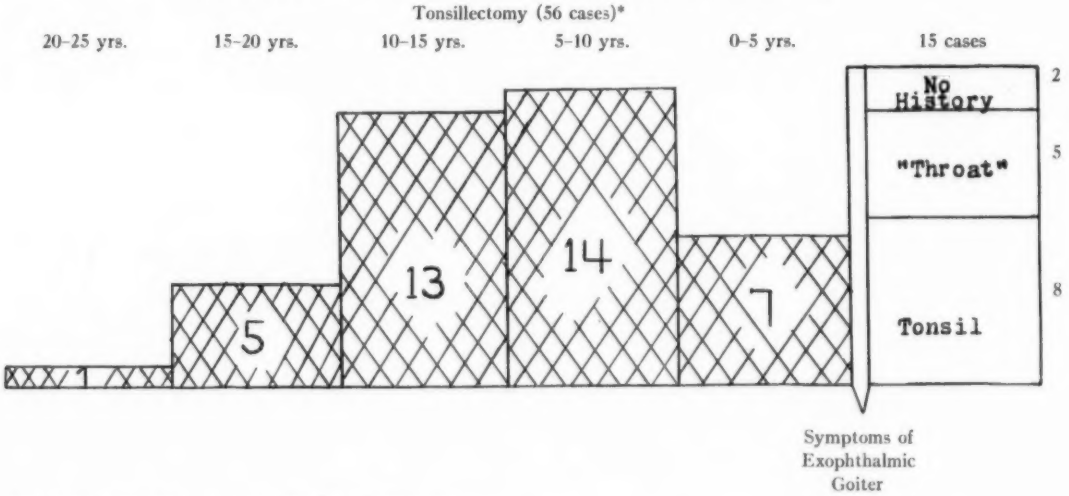
Recently, Rienhoff (16) injected the cervical lymphatics in dogs. Passing into a lymphatic vessel on the side of the neck, the injected material filled lymphatic spaces that bathe the thyroid, entered lymphatic vessels on the opposite side of the neck and reached the superior cervical (tonsillar) gland on the side away from the site injected. Thus, the lymphatic connection between tonsils and thyroid is intimate. As the thyroid is thought normally to drain outward into the lymphatics of the neck, one would have to assume, to advocate the infectious theory, that the flow may be reversed.

In further support of this theory, one might mention the frequent association of cervical adenitis with chronic tonsillitis and exophthalmic goiter. I have no figures to report but have been surprised at the frequency with which this condition occurs and the remarkable size these glands sometimes attain.

It has proved very difficult for observers to explain the "lymphoid hyperplasia" of the pharynx and cervical adenitis on the basis of hypersecretion of the thyroid gland. The symptom, exophthalmos, has also proved rather elusive to investigators. It is clear that repeated attacks of tonsillitis frequently precede the onset of exophthalmic goiter, while an acute pharyngitis or tonsillitis may seem to precipitate the disease. The analogy between this state of affairs and the relation between upper respiratory infections and rheumatic

TABLE 5

Analysis of time relation between tonsillectomy and onset of exophthalmic goiter



*No dates were given for 1 case having tonsillectomy.

fever is close. In the latter case, the pharyngeal infection is perhaps generally looked upon as a focus of the rheumatic infection rather

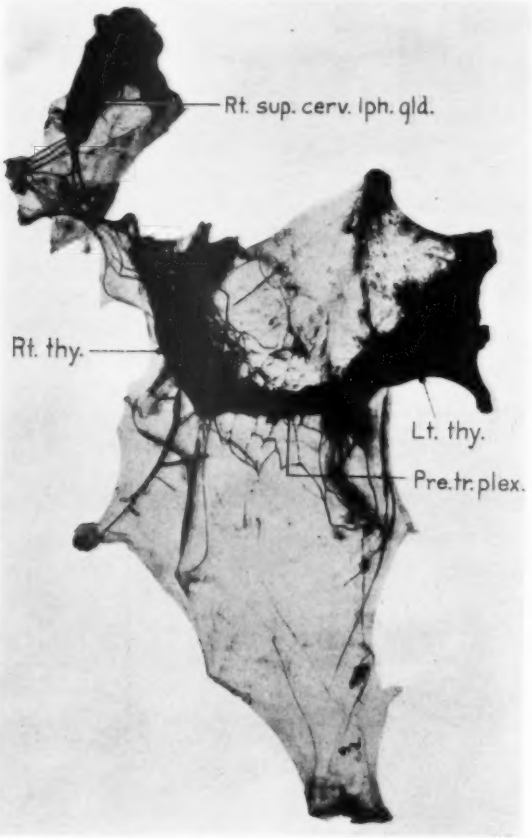


FIG. 1. Rienhoff's injection, showing lymphatics in dog's neck. Note intimate connection between the right superior cervical lymph gland and right thyroid. (Courtesy of Archives of Surgery.)

than as a precursor or sequel. The writer considers a similar conclusion the most reasonable explanation of the exophthalmic goiter problem: that, while upper respiratory infections frequently precede

or precipitate attacks of exophthalmic goiter, they eventually become a part of the disease itself. In other words, to the writer, exophthalmic goiter is not purely a thyroid disease, but an infectious disorder involving the pharynx, the thyroid gland and the communicating lymphatic system. An explanation of exophthalmos follows, from involvement of the cervical sympathetic system in the process.

In certain respects, it is difficult to escape the analogy that suggests itself between pharyngeal infections and rheumatic fever, on the one hand, and similar infections and exophthalmic goiter, on the other. Except in an occasional case, however, such as that of W. T. in the series of cases (*v.s.*), there is little to suggest that the causative agent of rheumatic fever, whatever that may be, is the same as that of exophthalmic goiter. The incidence of previous rheumatic fever in the series of exophthalmic goiter cases is only slightly higher than that shown by the control series. Moreover, exophthalmic goiter is uncommon in childhood, while rheumatic fever is most prevalent at that time of life.

The analogy seems to hold, however, in that tonsillectomy in many cases fails to prevent subsequent development of rheumatic fever and also of exophthalmic goiter. The comparison between rheumatic fever and exophthalmic goiter has been stressed for the purpose of drawing possible analogies between a disease (rheumatic fever) in which pharyngeal infections are generally admitted to play an important rôle, and exophthalmic goiter, in which such infections, though frequently remote, appear to be equally prevalent and probably important.

CONCLUSION

The incidence of tonsil infection in exophthalmic goiter is higher than it is in a mixed group of patients with tuberculosis, cancer and syphilis. The incidence is higher in each department of the comparison and higher than in any one of the three groups of "control" cases. On the other hand, the incidence in exophthalmic goiter is essentially the same as in rheumatic fever.

The importance of such infections must be determined for exophthalmic goiter as has been done for rheumatic fever. At present, there is danger that the once popular infectious theory of exophthalmic

goiter may be submerged and forgotten. The material herewith reported clearly supports such a theory, though final acceptance or disproof will depend probably upon the gradual accumulation of clinical findings. We have learned from experience of rheumatic fever that no significance can be attributed to the fact that tonsillectomy does not necessarily cure a condition with which tonsil infections are intimately related in an etiologic rôle. I have never seen tonsillectomy cure exophthalmic goiter, but I believe that this procedure helps to prevent recurrences.

The infectious theory has been obscured by the concept that infection in general may cause thyroid enlargement and that the thyroid may in this manner aid in combating infection. This may be true but there is no evidence that infections other than pharyngeal and "rheumatic" infections are concerned in the etiology of exophthalmic goiter. There is sufficient evidence in this one direction, however, to warrant serious consideration.

It is unnecessary to labor the question whether upper respiratory infections and lymphoid hyperplasia precede or follow the establishment of exophthalmic goiter. They are best explained as a part of the disease itself, establishing with cervical adenitis and thyroid hyperplasia a syndrome of infectious origin known as exophthalmic goiter.

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RESEARCHES ON TETANUS

VIII. At What Point in the Course of Tetanus Does Antitetanic Serum Fail to Save Life?[1]

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I

The wash-out experiments described in Papers V and VII of our series have shown that in any given case of experimentally induced tetanus it is only that fraction of toxin that has been fixed by the specifically reactive tissues of the body that is decisive for life or death, and it cannot be doubted that this statement holds as well for every instance of tetanus in man. Any of the toxin, over what has already been fixed upon these tissues, that may happen to circulate in the cardiovascular and lymphatic systems is no longer materially significant *quoad vitam*.

The wash-out procedure disclosed the fact that the timely injection of a large amount of antitetanic serum neutralizes fixed toxin and prevents it from exerting its usual fatal action. A striking example of the ability of the serum to save an animal from the inevitable and deadly consequences of even a large amount of fixed toxin is seen in the experiment with Dog X-16 (Paper VII), whose tissues had fixed, in the relatively short time of eight hours, 7.7 dog lethal doses of the toxin—an amount equivalent to 46.7 per cent of an intravenously injected total of 16.5 dog lethal doses.[2] As was stated in the protocol,

[1] A grant from the Carnegie Corporation of New York has made it possible for the authors to carry on a series of investigations on tetanus.

[2] We have found in the course of very numerous experiments extending over a period of five years that injection of 480 guinea-pig LD50's per kilogram invariably induces fatal tetanus in dogs, of medium weight and age, and have therefore arbitrarily chosen this figure as our lethal dose for dogs. This dose kills dogs, taken at random, in from 5 to 9 days. Neither a median nor a minimal lethal dose, but certainly larger than a statistically determined median lethal dose would

a large excess of antitoxin (65,450 U.S. units) was injected into the blood stream at the close of the experiment, when blood- and lymph-borne toxin had been removed and none but the fixed toxin had to be neutralized. From this time on the animal never gave the slightest evidence of tetanus or of discomfort of any kind; on the third day after the operation the animal began to take on weight and was in the best condition until it was killed by another dog seven weeks later; while the control animal, which had received the same large dose (16.5 dog L.D.'s) of the toxin, but which had not been bled and transfused, nor treated with antitetanic serum, succumbed to tetanus on the third day. Our new observation, that the antibody of tetanus can actually neutralize a fatal amount of fixed toxin, provided it be injected not later than at a time that will be defined later, is quite opposed to the belief of Courmont and Doyon, Roux and Borrel, Meyer and the many other writers who have regarded an action of this kind as being impossible.

The proponents of the nerve carriage theory of the toxin believe that the characteristic symptoms of tetanus, inclusive of the rigidity and contractures of local tetanus, are caused solely by the action of the toxin on central motor neurons. They also maintain that the toxin that has been fixed upon these neurons can no longer be neutralized by tetanus antitoxin because this principle is supposedly incapable, on account of the large size of its molecules, of reaching the neurons, either by way of the axis cylinders of motor nerves or by way of the circulation. The facts that were brought to light in our earlier papers (III, V and VII) have, however, made it clear that it is only by way of the blood stream that the toxin of tetanus as well as its antibody can reach the central nervous system.

At the close of our wash-out experiments we had animals in hand whose specifically reacting nervous centers had irreversibly fixed enough of the originally injected toxin to insure death, while all unbound toxin, except an immaterial residue, had been removed from their cardiovascular and lymphatic systems. When such wash-out

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be, it was adopted as a matter of convenience. We have always stated the dosage of the injected toxin in terms of standard guinea-pig units, so that our work can readily be checked by others without reference to our arbitrary dog unit.

animals, that had fixed a lethal amount of the toxin, were injected intravenously with a large amount of antitetanic serum, they showed no symptoms of tetanus and remained entirely well, while control animals that had received no serum succumbed to tetanus induced by their fixed toxin. This life-saving action of blood-borne antitoxin is made possible only because its molecules come into contact with and neutralize the toxin, that has been irreversibly fixed upon the central motor neurons. It will be shown later that the serum is no longer able to neutralize the toxin that has been fixed by the motor neurons, to speak only of these, if its injection be too long deferred.

The contention of those who maintain that an impenetrable brain-blood barrier prevents circulating antitoxin molecules from reaching and neutralizing fixed toxin cannot be reconciled with the facts that our experiments have brought to light. It is our purpose in the present paper to describe the factors that set so sharp a time limit to this neutralizing and life-saving action of antitetanic serum. For reasons that much be clearly evident these factors can be disclosed only by properly conducted experiments on animals that are susceptible to tetanus.

It will not be thought surprising that both tetanus toxin and its antibody can reach the motor neurons of the spinal cord, medulla and pons cerebri, by way of the blood stream and that the so-called brain-blood barrier is freely permeable to them, when it is borne in mind that both of these principles enter and escape from the lymph and blood capillaries with the greatest ease. For example, the permeability of the blood capillaries for the toxin is shown when this principle is injected directly into the substance of the anterior horn of the spinal cord, a region that is entirely devoid of lymphatic capillaries. When considerable quantities of the toxin are injected into this part of the cervical or lumbar cord, the innumerable blood capillaries surrounding the motor neurons constitute the sole pathway for the rapid removal of the non-fixed excess of toxin. The removal of the non-fixed excess by the blood capillaries proceeds so rapidly that not enough of it is left behind to diffuse upward and downward in the spinal cord. Attention was called to this point both in Paper VI (Firor and Jonas) and in Paper V, pages 366 and 367.

Elsewhere in the body the removal of toxin and of antitoxin from an injected area is effected both by the blood and lymph capillaries,

but often chiefly by the latter, particularly after subcutaneous injection at any point of the body or after intramuscular injection at multiple points.

The determination of the point in the course of an otherwise fatal descending or general tetanus, at which fixed toxin is no longer neutralizable by antitetanic serum, could have been established by means of a larger number of the time-consuming wash-out experiments with dogs, since the excess of unbound toxin present in the blood and lymph of these animals is easily removed. The experiments described in Papers V and VII of our series have shown that the amount of the toxin that has been fixed by the specifically reacting tissues of an animal is decisive for life or death, and that any additional amount of the toxin that may thereafter happen to be present in its blood and lymph is without significance in the further course of the disease. We have shown that certain animals that are highly susceptible to tetanus, such as the horse and sheep, succumb to a single lethal dose of the toxin, without giving evidence that any part of the injected dose circulates in the blood and lymph. In the various tissues of these animals, the dose of the toxin that just suffices to kill in the course of ten or twelve days, is contained in the fixed form only. Practical considerations, however, precluded the use of horses and sheep for the determination of the point at which a single lethal dose of the toxin is no longer neutralized by antitetanic serum.

The experiments described in Papers V and VII have shown very clearly that the bio-assays of the blood and lymph of dogs that have been injected at a definite time with a known number of fatal doses of the toxin enable us to determine the number of hours that elapse between the injection and the fixation, by these animals, of one or more fatal doses. With this information in hand it was just as possible to find the point at which fixed toxin is no longer neutralizable by antitetanic serum, by experimenting with injected but not washed-out dogs and monkeys, as with washed-out animals. Once a fatal dose, or more, of the toxin has been fixed in the body, any toxin that happens to circulate in the blood and lymph is as completely and easily neutralized by the injected antitetanic serum, as would be the case in an *in vitro* experiment. The results obtained in such less elaborate but equally conclusive experiments are to be found summarized in Tables I and II.

TABLE I

The treatment of toxin-injected dogs with antitetanic serum

DOG	WEIGHT	DOSE IN DOG L.D.'s	ROUTE OF INJECTION	UNITS OF ANTITOXIN INJECTED PER KILOGRAM	TOTAL NUMBER OF UNITS OF ANTITOXIN INJECTED[9]	INTERVAL BETWEEN INJECTION OF TOXIN AND INJECTION OF ANTITOXIN	INTERVAL BETWEEN INJECTION OF TOXIN AND APPEARANCE OF FIRST DISCERNIBLE SYMPTOMS OF TETANUS	REMARKS
	kg.							
X-11	8.0 (control)	3.0	Intravenous				48 hours	Died of tetanus in 5 days
Z-26	9.0 (control)	3.0	Intravenous				41¾ hours	Died of tetanus in 61½ hours
Z-21	12.0	3.0	Intramuscular, right front leg	173	2,080	8 hours	Local tetanus in 70 hours	No symptoms of descending tetanus appeared. Recovered
Z-71	9.1	3.0	Intramuscular, right front leg	8,520	77,500	48 hours	Local tetanus of injected leg in 48 hrs.	No symptoms of descending tetanus appeared. Recovered
Z-23	12.5	3.0	Intravenous	173	2,160	9½ hours		No symptoms of tetanus appeared. Remained well
Z-14	7.5	3.0	Intravenous	176	1,320	12 hours	96 hours	Moderately severe descending tetanus. Recovered
Z-13	7.8	3.0	Intravenous	169	1,320	23½ hours	48 hours	Died of tetanus in 106 hours
Z-12	10.3	3.0	Intravenous	1,750	18,000	24½ hours	96 hours	Mild cephalic tetanus. Recovered
Z-25	10.5	3.0	Intravenous	1,720	18,020	24 hours	96 hours	Mild descending tetanus. Recovered
Z-29	7.6	3.0	Intravenous	3,460	26,350	30½ hours	48 hours	Mild descending tetanus. Recovered
Z-32	7.0	3.0	Intravenous	6,850	47,940	36 hours	94½ hours	Mild descending teatnus. Recovered
Z-33	5.25	3.0	Intravenous	10,300	54,060	40 hours	103 hours	Mild descending tetanus. Recovered
Z-9	7.0	3.0	Intravenous	189	1,320	43¾ hours	48 hours	Died of tetanus in 53 hours

TABLE 1—*Concluded*

Z-27	9.0	3.0	Intravenous	6,570	59,090	43¼ hours	43¼ hours	Died of tetanus in 65¾ hours
Z-8	11.6	3.0	Intravenous	1,710	19,840	48 hours	72 hours	Died of tetanus in 10 days
Z-11	5.7	3.0	Intravenous	1,690	9,600	48 hours	48 hours	Died of tetanus in 53 hours
Z-37	8.0	3.0	Intravenous	14,400	115,090	48 hours	48 hours	Died of tetanus in 90 hours
Z-38	7.7 (control)	10.0	Intravenous				48 hours	Died of tetanus in 72 hours
Z-72	9.1 (control)	10.0	Intravenous				23 hours	Died of tetanus in 70 hours
Z-57	12.7 (control)	10.0	Intravenous				60 hours	Died of tetanus in 4 days
Z-39	8.4	10.0	Intravenous	5,780	48,620	4 hours		No symptoms of tetanus appeared. Remained well
Z-15	11.8	10.0	Intravenous	559	6,600	12 hours		No symptoms of tetanus appeared. Remained well
Z-48	12.7	10.0	Intravenous	2,610	33,150	24 hours	47⅓ hours	Died of tetanus in 53⅓ hours
Z-49	10.5	10.0	Intravenous	6,640	69,700	24 hours		Killed by another dog 5 days later. No symptoms of tetanus observed
Z-64	8.0	10.0	Intravenous	8,750	70,000	24 hours		No symptoms of tetanus appeared. Remained well
Z-50	11.2	10.0	Intravenous	6,250	70,000	36 hours	36 hours	Died of tetanus in 70 hours
Z-55	5.7	10.0	Intravenous	6,320	36,000	30 hours	70 hours	Died of tetanus in 5 days
Z-62	8.20	10.0	Intravenous	8,540	70,000	30 hours	48½ hours	Died of tetanus in 6 days
Z-69	9.0 (control)	100.0	Intravenous				25½ hours	Died of tetanus in 48 hours
Z-70	9.1	100.0	Intravenous	8,520 3,410	77,500 31,000	3½ hours 24½ hours		No symptoms of tetanus appeared. Remained well
Z-68	7.05	100.0	Intravenous		77,500	6 hours	29¾ hours	Died of tetanus in 48 hours
Z-65	9.3	100.0	Intravenous	8,340	77,500	12 hours	48 hours	Died of tetanus in 4 days
Z-63	7.95	100.0	Intravenous	8,810	70,000	20 hours	48 hours	Died of tetanus in 4 days

* The amounts of antitoxin employed are stated in terms of U. S. units. The serum was injected intravenously in each instance.

TABLE II

Limitations of treatment with antitetanic serum of tetanus in monkeys

DATE	MONKEY	WEIGHT	AMOUNT OF TOXIN INJECTED (GUINEA-PIG UNITS PER KG.)	SITE AND MANNER OF INJECTION	NUMBER OF UNITS OF TETANUS ANTITOXIN INJECTED PER KILOGRAM*	TOTAL NUMBER OF UNITS OF ANTITOXIN INJECTED	INTERVAL BETWEEN INJECTION OF TOXIN AND INJECTION OF ANTITOXIN	INTERVAL BETWEEN INJECTION OF TOXIN AND APPEARANCE OF LOCAL TETANUS	INTERVAL BETWEEN INJECTION OF TOXIN AND APPEARANCE OF FIRST SYMPTOMS OF DESCENDING TETANUS	FATE OF ANIMALS AND REMARKS
1938		*kg.*								
Feb. 2	K-441 (control)	4.5	4.0	Left gastrocnemius muscle at ten separate points				47½ hours	66½ hours	Feb. 4: Local tetanus: flexion of toes of left foot. Feb. 5: Left leg drawn up. General tetanus, difficulty in opening mouth. Feb. 6: Animal prone. Opisthotonos, stiffness of truncal musculature. Respirations labored. Feb. 7: Dead.
Feb. 7	G-15	4.3	4.0	Left gastrocnemius muscle at ten separate points	4,280	18,200	45 hours	45 hours		Feb. 9: Local tetanus: flexion of toes of left foot. Resistance to passive flexion of ankle. Antitetanic serum given. Feb. 11: Animal is lively. No evidence of general tetanus. Left leg stiff in extension. Feb. 13: Edema of left leg owing to prolonged maintenance in a dependent position.† Feb. 15: Same. Leg stiff and swollen. Feb. 21: Very active. Condition of leg unchanged.
Jan. 31	K-518	4.0	4.0	Left gastrocnemius muscle at one point	4,450	16,800	96 hours	47 hours	96 hours	Feb. 2: Toes of left foot are fixed in flexion. Spares foot while running. Definite resistance to passive flexion of ankle. No symptoms of descending tetanus. Feb. 4: Left leg is useless, drawn up against body. Cannot open mouth freely. Antitetanic serum injected. Feb. 5: Unable to eat because of advanced trismus. Left leg flexed at knee and hip. Some difficulty in using the left shoulder. Feb. 6: Trismus complete. Profuse salivation. Opisthotonos. Fixation of muscles of respiration. Marked resistance to passive flexion of left shoulder. Left leg extended. Reflex motor symptoms: exaggeration of opisthotonos when a loud noise is made. Feb. 7: Dead.

Jan. 28	K-1 (control)	2.9	8.0	Intravenous					43½ hours	Jan. 30: 10:30 A.M.: Malaise. Cannot open mouth beyond 0.5 cm. Respirations rapid and shallow. Some stiffness of neck muscles. 10:30 P.M.: Lying on side. Trismus complete. Cervical opisthotonos. Definite stiffness of fore limbs. Falls on face when crawling about. Occasional light tetanic convulsions brought on by touching skin. Jan. 31: Dead.
Feb. 14	K-561	5.15	8.0	Right gastrocemius at ten separate points	4,560	23,250	71 hours	54 hours	71 hours	Feb. 16: Toes of right foot flexed. Feb. 17: Beginning trismus. Cervical rigidity. Antitetanic serum injected. Feb. 18: Unable to eat. Muscles of trunk rigid. Right leg stiff in extension. Animal can no longer crawl about. Feb. 19: Dead.
Feb. 11	K-2	3.9	40.0	Intravenous	5,960	23,250	26½ hours	77 hours	46 hours	Feb. 12: No symptoms of tetanus. Antitetanic serum injected. Feb. 14: Malaise. Movements slow and awkward. Serum injected. Feb. 17: Slight trismus. Definite cervical rigidity. Feb. 20: Condition improving. Opens mouth freely.
					3,940	15,500	77½ hours			
Feb. 14	K-572‡	4.67	1.0	Muscles of pharynx and larynx and muscles of mastication				23 hours		Feb. 14: Animal anesthetized with nembutal. Toxin injected perorally into muscles of pharynx and larynx at multiple points. Toxin also injected at many separate points into the muscles of mastication. Feb. 15: Can open mouth freely but has difficulty in swallowing. Feb. 16: Beginning trismus. Dyspnoea. Fluids regurgitated through the nose and mouth. Feb. 18: Respirations slow and labored. Rigidity of muscles of jaw and neck. Feb. 19: Moribund. Killed by intracardiac injection of air.

* All of the injections of antitoxin were given intravenously.

† When the affected leg was elevated for a number of hours, with the monkey under nembutal anesthesia, the edema rapidly subsided.

‡ In this experiment a fatal local tetanus of the head and neck resulted from the intramuscular injection, in the manner described, of 1 guinea-pig unit of the toxin per kilogram (0.25 monkey L.D.). The injection of antitetanic serum after the appearance of muscular stiffness would not have saved the life of this monkey, since antitoxin is powerless to undo the stiffness of toxin-poisoned muscles; the increasing stiffness of the muscles of the larynx, pharynx and the jaws is sufficient to insure death.

II. TREATMENT OF TOXIN-INJECTED ANIMALS WITH ANTITETANIC SERUM[3]

Two classes of animals were employed, the dog and the monkey, the former representing the class that is more or less resistant to the toxin, and the latter the highly sensitive group that includes the horse and man.

Experiments with Dogs

We have summarized in Table I the results of three series of experiments with dogs. The animals of the first series received 3 lethal doses, those of the second, 10, and those of the third, 100.

First series. It was necessary to determine for each series the time at which the first definite symptoms of tetanus appeared in control animals, after the injection of these doses of the toxin. The control animals of the first series, which had received 3 lethal doses, showed definite symptoms of tetanus in about 48 hours, and succumbed three to five days after the injection.

Effect of antitetanic serum on local tetanus. Two animals of the first series, Z-21 and Z-71, that were to be treated with antitetanic serum, received the toxin intramuscularly into the right foreleg. Eight hours later, when there were as yet no symptoms, Dog Z-21 received intravenously 173 units of antitoxin per kilogram of body weight; nevertheless, 70 hours later a marked local tetanus appeared in the injected leg, despite the presence of a considerable amount of circulating antitoxin. The antitoxin, however, prevented the appearance of other symptoms of tetanus. Dog Z-71 had local tetanus within 48 hours, at which time antitetanic serum amounting to 8,516 units per kilogram was injected intravenously. No other symptoms of tetanus appeared, although, as this paper goes to press, six weeks from the date of the administration of the toxin, local tetanus of the leg is still present.

Effect of antitetanic serum on animals injected intravenously with the toxin.[4] The remaining animals of the first series received the toxin intravenously and naturally in none of them could a local tetanus, in the true sense of the term, make its

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[3] For so generously contributing the large amounts of concentrated antitoxin used in the experiments described in this paper, we are greatly indebted to Dr. William A. Feirer, Medical Director of the Sharpe & Dohme Laboratories, and to Dr. John T. Anderson, Medical Director of the Biological Laboratories of E. R. Squibb & Sons.

[4] The quantities of antitetanic serum employed in these experiments represented from 100 to 10,000 times the amounts required to neutralize the injected toxin. It is not surprising therefore that even the animals that we failed to save should have retained in their blood and lymph vascular systems a large excess of free antitoxin.

appearance. A few typical cases will serve to show the effect of delaying treatment with antitetanic serum. In Experiment Z-14, where 12 hours elapsed between the injection of the toxin and that of the antitoxin, the period of incubation was 96 hours, as compared with 48 hours in the control. A moderately severe descending tetanus followed, but the animal eventually made a complete recovery.

Dog Z-13 was not injected with antitoxin until 24 hours after the injection of the toxin; it died of tetanus in four days. The failure to save its life is accounted for by the employment of a dose of antitoxin (169 units per kg.) that was too small for an experiment with so long a waiting period as 24 hours. That this was the case was clearly shown by the results of Experiment Z-12, where the amount of toxin injected per kilogram and the waiting period were the same as in Z-13, but where the dose of antitoxin was ten times larger. In Z-13 there was an interval of 96 hours between the injection of the toxin and the appearance of discernible symptoms of tetanus; the mild cephalic symptoms that developed cleared up in ten days.

As the interval between the injection of the 3 lethal doses of toxin and the injection of the antitoxin increased, increasingly larger amounts of serum had to be employed. We failed, however, to save our animals when the administration of even very large quantities of the serum was delayed beyond the 40-hour interval, that is to say, until after the first symptoms of descending tetanus had appeared. The use of the serum so late as the 40-hour period, at a time when no symptoms could be detected, did not, however, prevent the appearance of a mild descending tetanus. This is well illustrated in the experiment with Dog Z-33, which received 10,300 units of antitoxin per kilogram.

The ineffectiveness of serum when given coincidently with the appearance of definite manifestations of general tetanus—erection of the ears, furrowing of the brows and narrowing of the palpebral fissures—is well shown in Experiments Z-27, Z-11 and Z-37. All of these animals died of tetanus in less than four days. The injection of so large an amount of antitoxin as 14,400 units per kilogram into one of these animals (Z-37) exerted not the slightest favorable effect on the course of the disease.

Second series. In the second series of experiments, 10 dog lethal doses were injected. The first definite symptoms of tetanus appeared in the control animals in 48 hours, and in one instance in 60 hours; death occurred on the third or fourth day. The prophylactic action of tetanus antitoxin was effective only when the serum was administered within less than 30 hours after the injection of the toxin. In other words, when the giving of the serum was delayed beyond the middle of the period of incubation, the animals of this second group succumbed to tetanus as soon as those that had not received serum. Even early in the incubation period, these animals could not be saved unless very large amounts of serum were given. Thus, while 2,610 units per kilogram failed to save Dog Z-48 when injected at the 24-hour period, 6,640 units proved effective in another animal, Dog Z-49.

Third series. As the interval between the injection of toxin and the injection of serum increases, an increasingly larger amount of the serum must be injected in order to save life, if, indeed, this can still be done. When, however, the injection of the antitetanic serum is delayed until unmistakable signs of descending tetanus follow the injection of one or more lethal doses of the toxin, life cannot be saved, even by the use of the largest possible amounts of serum. On the other hand, irrespective of the amount of toxin that has been injected, life can still be saved by employing considerable amounts of the serum, if these are injected soon enough after the administration of an excessive amount of the toxin—at a time when absolutely no symptoms of general tetanus are in evidence. This statement is well illustrated by experiments in which 100 dog lethal doses of the toxin (48,000 guinea-pig units per kg., or approximately 12,000 times the kilogram-lethal dose for the horse, the monkey and probably also for man) were injected, and in which life was saved by the use of excessive quantities of serum within five hours after giving the toxin.

Control dogs injected with 100 lethal doses (48,000 guinea-pig units per kg.) showed symptoms of tetanus in 24 hours and died in 48 hours. It was possible to save these animals only when the serum was given within less than six hours after injection of the toxin. This is shown by Experiments Z-68 and Z-70, where the waiting periods were 6¼ and 3½ hours, respectively. A large amount of antitetanic serum was employed in both instances; the first dog (Z-68) died of tetanus in less than 48 hours, while the second one (Z-70) survived without having shown any symptoms of the disease. In animals such as Z-70, where so many as 40 out of 100 lethal doses are fixed or otherwise disposed of, the antitoxin molecules of the serum are still able to reach and neutralize the fixed toxin. When, as in Experiment Z-68, the serum is injected too late to save life, the period of incubation may nevertheless be prolonged; the antitoxin molecules can neutralize large amounts of fixed toxin during the first stage of the incubation period, but are quite powerless to do so at a later time, when the fixed quantum of the toxin may have undergone such changes as to preclude its continued neutralization.

From the three series of experiments the following interesting facts emerge: (1) Antitetanic serum exerts a prophylactic effect when injected into dogs poisoned with 3 to 100 lethal doses of the toxin. When 3 lethal doses are injected, the giving of an appropriate amount of antitetanic serum will save life at any time during the period of incubation; but when more than 3 lethal doses are injected, the life-saving action of serum does not extend throughout the entire incubation period. For example, animals injected with 100 lethal doses can be saved only up to about the fifth hour of the period of incubation. (2) As the interval following the injection of the toxin is prolonged, the amount of antitetanic serum required to save an animal rapidly

increases. If sufficient data were available the time-concentration curve would, no doubt, be found to be exponential in character. (3) The life-saving power of antitoxin when injected into toxin-poisoned animals is ultimately dependent upon its ability to neutralize fixed toxin. It has been shown by our wash-out experiments that any free toxin present in the blood and lymph is really of no further significance, once a lethal dose has been fixed. An appropriate amount of intravenously injected antitetanic serum would necessarily neutralize any free circulating toxin, while it is being carried to the fixed toxin. This occurred in the animal experiments described in this paper, in which the freely circulating toxin had not been removed from the blood and lymph vascular systems by the wash-out procedure.

Experiments with Monkeys[5]

In the third year of the World War Sherrington published observations concerning the effect of antitetanic serum upon experimentally induced tetanus in monkeys. While his experiments seemed to show that the intrathecal and the intrabulbar routes of injection were more efficacious than the intravenous route, clinical experience does not point to a similar superiority of these methods in cases of human tetanus. In our experiments with monkeys, which were carried out with the intention of defining as sharply as possible the time limits within which tetanus antitoxin is capable of exerting a life-saving action in animals poisoned with one or more lethal doses, we have injected the serum intravenously in every instance.

Sherrington injected into the gastrocnemius muscles of monkeys an amount of tetanus toxin that led to the development of local tetanus of the injected leg in 30 to 36 hours, and of the first symptoms of descending or general tetanus in 72 hours; the death of the control monkeys occurred on the third to the fifth day. The time that elapsed between the injection of the toxin and that of the serum varied from 47 to 78 hours. A careful study of his tables reveals that, when the injection of antitetanic serum (2000 U.S. units per

[5] In carrying out our experiments with monkeys it was of great advantage to us that we could avail ourselves of the extensive knowledge and the generous cooperation of Dr. Carl G. Hartman, of the Department of Embryology of the Carnegie Institution of Washington.

kilogram) was delayed until 74 hours after injection of the toxin (at which time unmistakable symptoms of descending tetanus were in evidence), none of the monkeys could be saved; animals treated after this interval of time usually died at the same time as the controls, but occasionally lived a day or two longer. Sherrington succeeded in saving only those monkeys that showed no other symptoms except the local tetanus that always follows an intramuscular injection of a sufficient amount of the toxin.

Our experiments with monkeys are summarized in Table II. The animals were adults of the species, *Macaca mulatta*, whose average weight was approximately 4,000 grams. We found that the lethal amount of toxin that suffices to kill such monkeys in five to nine days is 4 guinea-pig median lethal doses per kilogram of body weight. Thus, it is evident that monkeys of this species are as sensitive to tetanus toxin as the horse, whose kilogram-lethal dose is also 4 guinea-pig units.

Definite symptoms of general tetanus were observed in our first control monkey, which received intramuscularly 4 guinea-pig units of toxin per kilogram, in 66½ hours, and death occurred on the fifth day. A second control, injected intravenously with 8 guinea-pig units per kilogram, showed symptoms of descending tetanus in 43 hours and died in 65 hours. The march of symptoms in the two monkeys was very similar to that observed in the natural disease in man.

When such doses of the toxin were injected intravenously, there appeared, in two or three days, loss of appetite and difficulty in swallowing and in opening the mouth. A few hours later rigidity of the cervical muscles was noticed, together with a beginning opisthotonos; the animal no longer leaped about the cage in its usual lively manner. On the fourth day there developed complete trismus, marked opisthotonos and widespread muscular stiffness, which involved chiefly the muscles of the head and trunk, the extremities being affected but slightly up to the very last. Extensive involvement of the arms and legs also occurs rarely in the general tetanus of human beings. On the other hand, the tactile reflex motor phenomena that play so important a part in human tetanus, are much less conspicuous in monkeys. Sherrington, in his careful study of more than 100

toxin-poisoned monkeys, makes no mention of reflex motor tetanus, and we succeeded in demonstrating it on only two or three occasions.

When a lethal dose is injected into the gastrocnemius muscles of monkeys a double form of tetanus results. First, a local tetanus of the injected extremity develops, the first symptom of which is flexion of the toes. This is soon followed by extension of the ankle and partial flexion of the knee and hip. Finally, the tetanized leg becomes rigid in extension. On the third or fourth day the first symptoms of the fixation of blood-borne toxin by the voluntary muscles and the motor neurons make their appearance—malaise, dysphagia, salivation, trismus, and stiffness of the cervical muscles.

As stated in our protocols, monkeys were injected with 4.0, 8.0 and 40.0 guinea-pig units of toxin per kilogram of body weight, and at varying intervals thereafter were given large amounts of antitetanic serum. The results of these experiments, which are summarized in Table II, agree in every way with those obtained in our similar studies with dogs. Monkeys poisoned with one or more lethal doses could be saved only when the serum was administered before the appearance of the first discernible symptoms of general or descending tetanus. When the serum was injected very soon after the appearance of local tetanus, general tetanus did not occur (G-15). The local symptoms nevertheless progressed until the injected leg was completely stiffened.

In Experiment K-2, a monkey was injected intravenously with 10 lethal doses (40 guinea-pig units per kg.), and samples of blood were collected 4 hours and 20 minutes and 24 hours later, for bioassay of the amount of unbound toxin in the blood. Assuming that at the 4-hour period there was an equipartition of toxin between the blood and lymph vascular systems, and that the blood of the monkey amounts to 8 per cent of its body weight, we find that in the relatively short time of $4\frac{1}{3}$ hours the animal had fixed and otherwise disposed of 3 out of the 10 lethal doses originally injected. But the assay of the 24-hour blood showed that all but a trace of the toxin had disappeared from the blood stream. In its ability to bind several lethal doses of toxin the monkey differs from some of the other sensitive animals, such as the horse and the sheep, whose tissues are endowed with the ability to fix little if any more than one full lethal dose.

It resembles in this respect the relatively resistant dog, whose tissues, as we have shown in Paper VII, are able to fix and dispose of many lethal doses.

Twenty-six hours and 20 minutes after the injection of the toxin, Monkey K-2 was injected intravenously with antitetanic serum (5,960 U.S. antitoxin units per kg.). Less than 48 hours later, however, unmistakable symptoms of descending tetanus appeared—malaise, awkwardness and slight difficulty in opening the mouth. Another injection of serum (3,940 units) was therefore given. With the exception of stiffness of the cervical muscles, which was detected three days after the second injection of the antitetanic serum, no further progression of symptoms occurred and the animal made a slow but complete recovery.

III

1. We may now consider the question, to what extent should the failure of antitetanic serum to save the lives of animals that have fixed a lethal dose of the toxin influence physicians in estimating its value in the treatment of human tetanus. It need not be pointed out how greatly conditions differ in the case of patients that come to our hospitals in the various stages of tetanus, from those encountered in the well controlled instances of experimental tetanus. It is well known that in the days before the discovery of tetanus antitoxin some 30 per cent and more of all cases of tetanus recovered, and it is also well known that the authors of numerous statistical studies have concluded that there is no evidence that the death rate of tetanus has been lowered as a result of the extensive use of antitetanic serum, once the symptoms of general tetanus, as distinguished from the local type, are well established. Nevertheless, the time has not come when we can say with any degree of certainty that the administration of the serum can be dispensed with in human tetanus, unless, indeed, the patient comes to our attention at a time when he is moribund and intervention of any kind is plainly useless. The decision whether to use antitetanic serum, or not, must be made without delay at the time of the first examination of the patient. Who, in the present state of our knowledge, will venture to decide, at the time the patient first comes to hand, whether his tissues have absorbed and fixed a full

lethal dose of the toxin, or three quarters or a half of this amount? The reason that as many as 30 per cent of all cases of human tetanus finally recovered, in pre-antitoxin days, is found in the facts that have been brought to light by injecting animals with graded doses of the toxin. Experiments of this kind support the following statement: When the amount of the toxin that is fixed by the tissues falls short of the full lethal dose, even though it may amount to three quarters of a lethal dose, recovery finally takes place, with or without antitetanic serum. We have, however, at the moment no definite test that enables us to decide, in an hour or less, whether, in any given case of human tetanus, a lethal dose of the toxin has been fixed at the time the patient comes to our attention. As is well known, the later course of this insidious disease is generally unpredictable. This holds true even for instances in which the period of incubation is as long as twelve to fourteen days or more. Many instances are cited in the literature of such patients, who appeared to have every chance of life, but in whom there occurred a sudden and fatal exacerbation of symptoms, because of a renewed production of the toxin in a concealed locus of infection.

Before the antitetanic serum was used, cases of this kind always died, sooner or later, of tetanus. Since today, all patients, irrespective of the length of the period of incubation, are injected with the serum, some therefore survive that might have died from the deadly effects of a subsequently produced charge of toxin. It would certainly not be permissible for a physician to make an offhand decision, in a case with an ascertained incubation time of twelve or more days, that the employment of antitetanic serum can be dispensed with on the assumption that his patient has every chance of recovering without it.

But there is one particular type of human tetanus, whose final outcome cannot be foretold, but in which the physician would not be justified in underestimating the extraordinary life-saving power of the timely employment of the serum. We have in mind cases that come to the physician with symptoms of a purely local tetanus of one of the extremities, for example, that do not show the slightest signs of tactile reflex symptoms. Here we may have a relatively long period of incubation, and here the toxin elaborated at the point

of infection first reaches, *by way of lymphatic net-works, and not by way of the blood stream*, certain groups of muscles in the neighborhood of the infected area. These instances of a prior local tetanus serve to illustrate the direct peripheral action of the toxin upon voluntary muscles, as distinct from its action upon central neurons. The literature of tetanus contains many instances in which a purely local tetanus of an extremity, or of a few groups of muscles, constituted the only evidence of the disease,[6] and it has often been pointed out that this form of tetanus occurs more frequently than one would suppose.[7] The contractured muscles return to their normal state in the course of several weeks or of many months.

Let us suppose that local tetanus is present in one of the extremities, but that there is as yet no evidence that a fatal amount of a gradually elaborated toxin has been fixed by the motor neurons of the neural axis. To these neurons the toxin can be carried only by way of the circulation. That a well established local tetanus may be followed in a few days by a fatal, descending or blood-borne tetanus (which is solely due to the transfer to central neurons, via the blood stream, of a lethal amount of the toxin), is clearly proved by numerous case histories of the past.

No fact is more clearly established, as is shown by the protocols of our experiments on monkeys and dogs, than that these animals can be saved from the effects of one or more fatal doses of the toxin, if a sufficient quantity of antitetanic serum is injected intravenously at a time when the only symptoms are those of a purely local tetanus, and when there is as yet no determinable evidence that a fatal, superimposed, descending tetanus is in the making.

In endeavoring to save human beings with a persistent local, and a later descending tetanus, we cannot now be certain, as we are in animal experiments, whether a full lethal dose or less has been fixed at the time when the antitetanic serum is to be injected. Despite our ignorance in respect to this fact, we nevertheless incline to the belief, in view of the past history of tetanus and in view of our success in saving animals under the conditions described in our protocols, that the injection of the serum into patients with this type of tetanus,

[6] See Paper IV, pages 360–363.

[7] See Klemm: *Deutsche Ztschr. f. Chirurgie*, 1896, xlii, 453.

at a stage comparable to that of our experiments, will save many of them, even when a full lethal dose of the toxin has been absorbed.

Of the dozen and more cases that we have had the privilege of studying in the hospitals of Baltimore in the past three years, we recall only two in which local tetanus was a well marked prior symptom. In one of the two cases the site of infection was in the crushed distal phalanx of the right ring finger; the local tetanus that developed in the interossei and lumbricales caused the fingers to be spread apart in extension. The other case, which is described in Footnote 8, was an example of local tetanus of the head. Although these patients later showed symptoms of descending tetanus, they recovered following the use of antitetanic serum. We cannot, however, state with any degree of confidence that the serum saved their lives, for we had no way of determining in either case whether a lethal amount of toxin had been fixed upon the tissues before the serum was given or whether a lethal dose was subsequently produced at the site of infection, only to be neutralized by the circulating antitoxin.

While we are discussing the conditions under which life may be saved by timely injections of antitetanic serum, it may be well to call attention to another variety of local tetanus that often responds favorably to such injections. This variety is known as head, or cephalic tetanus, and it is here that some of the finest examples of local tetanus are seen. If the seat of the infection is situated on one side of the face, the facial and the masseteric muscles of that side are the first to become rigid; if the lesion is medially situated, the muscles of both sides of the face and head will be equally affected. After an extensive wound of the scalp the resulting muscular rigidity and contracture may involve the ocular, facial, masticatory, pharyngeal, laryngeal and neck muscles to an equal degree on both sides. In the classical examples of head tetanus the rigidity of these muscles generally develops gradually, over a number of days. In benign cases, more particularly those in which the pharyngeal and laryngeal muscles remain unaffected, no further symptoms may develop. However, after an extreme local tetanus appears in the pharyngeal and laryngeal muscles there is little chance of recovery.[8]

[8] In severe cases of head tetanus in which the muscles of the pharynx and the larynx are chiefly involved, tracheotomy, when carried out in conjunction

There are many instances of head tetanus with a more prolonged incubation period, in which the later, superimposed and generalized reflex tetanic symptoms appear only very slowly, or not at all, as was remarked by Klemm (1889) in his studies on this form of the disease; and that careful clinical observer, Brunner, whose work on the experimental production of head tetanus in animals is well known, stated that the locally poisoned muscles, in the head tetanus of man, may be entirely free from centrally induced "tetanic cramps"; and he accordingly used the phrase, "localized stationary tetanus," as adequately descriptive of head tetanus.[9] In cases of this kind, the early injection of an appropriate amount of antitetanic serum, which should be considerably larger than the usual prophylactic dose, may prevent the further fixation of newly produced toxin and thus save life.[10] There are, however, many other cases of head tetanus, that do not remain in this state of a "localized stationary tetanus," free from centrally induced "tetanic cramps." Here, an additional type of tetanus, induced by blood-borne toxin, appears *when more toxin is produced at the site of infection than can be fixed by muscles of*

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with the administration of antitetanic serum, may sometimes prove to be a life-saving measure.

[9] See Paper IV of our series for references to papers of Klemm, Brunner and others on local tetanus.

[10] See, for example, the classical case described by Klemm in 1896, which deserves careful study. The local tetanus developed very slowly, while the patient was under observation; there was a period of three or four days, before the laryngeal and pharyngeal muscles had become locally tetanized, when the patient could have been saved by a liberal amount of antitetanic serum. Three years ago we observed a case very much like Klemm's, with a stellate laceration of the scalp in the occipital region, that had not received a prophylactic injection of antitoxin, in spite of the existence of trismus and some difficulty in swallowing and rigidity of the neck muscles at the time of admission, which symptoms were interpreted by us as due to a local tetanus in consequence of lymphatic carriage of the toxin. The administration of a total of 300,000 units of tetanus antitoxin intravenously and intramuscularly, together with the usual treatment with sedatives, prevented the later appearance of all but the mildest symptoms of a descending tetanus, and led to complete recovery. The patient had been admitted on the 11th of June, 1935, and was discharged on the 13th of July. In the interval the locally poisoned muscles gradually lost their stiffness, and when the patient left the hospital on July 13, the muscular rigidity had entirely disappeared.

the face, head and neck. This excess of the toxin is distributed throughout the body, via the blood stream. The muscles that are not in a state of local tetanus, and the central neurons, are now supplied with an effective amount of the toxin. If the excess amounts to a lethal dose or more, the neurons will fix their own lethal quantum of it.

Before the discovery and use of antitetanic serum all the cases of head tetanus of this kind terminated fatally. We are inclined, however, to believe that many cases of this conjoint type, of an earlier head and a later more generalized tetanus, that came under observation at a time when there was, as yet, no evidence of a highly reflex state, caused by the fixation of a fatal amount of the toxin upon central neurons, could have been saved by the injection of antitetanic serum at this time. Justification for this belief is found in the protocols of experiments with dogs and monkeys, that had been injected intramuscularly with one or more lethal doses of the toxin in such a manner as to produce a prior local tetanus. These animals were saved by the intravenous injection of large amounts of antitetanic serum, when there were as yet no symptoms of the disease except those due to local tetanus.

If we admit, as we must, in view of numerous statistical reports, that little if anything has been accomplished in a therapeutic way by the specific treatment of serious cases of human tetanus, it does not follow that we can dispense with antitetanic serum, or even that we are justified in employing it less freely than we are now doing.

The statistician deals with data concerning the number of people that have died of tetanus after treatment with antitetanic serum and the number that have recovered, the length of the incubation period, and other related points. The physician may be fully convinced of the correctness of the statistician's conclusion that the serum is devoid of curative value in the true sense of the term.[11] The

[11] Golla (1917), in his valuable statistical studies on tetanus, under the heading, *The Curative Action of the Serum*, makes the following statement: "To sum up, there appears no evidence of any appreciable diminution of the mortality of tetanus to be derived from an analysis of the statistics of incidence and mortality when allowance is made for the modification of the disease due to prophylaxis."

Huntington, Thompson and Gordon (1937), in a similar statistical analysis, state that "relatively little has been accomplished in the specific treatment of tetanus."

greater number of tetanus cases, however, come to the physician's notice in the early stages of the disease, at a time therefore when he still has the opportunity to avail himself of the possible life-preserving action (as opposed to the curative action) of suitable amounts of the serum. There are unfortunately no statistical data at his disposal, at this time when the events of tetanus are in the making, that will enable him to conjecture with some degree of probability which of his cases can be saved by the use of the serum and which not. He must therefore use it in all cases.

The uncertainty of the outcome of untreated cases, or even of cases treated with antitetanic serum, does not absolve us from the obligation of making use of the prophylactic action of the serum, in spite of its limitations. Because of our ignorance in respect to the factors involved in the earliest stages of human tetanus, particularly whether the toxin is being slowly elaborated at the site of infection or more rapidly and in such large amounts as to furnish many lethal doses, we must continue to employ the antitetanic serum in all cases of the disease, at the earliest possible moment, in order to save that limited number that we have every reason to believe, on the basis of our animal experiments, can be saved. Furthermore, a study of the literature of the tetanus of pre-antitoxin days brings to light many cases, more particularly those in which local tetanus was a prior symptom, that could surely have been saved by a timely injection of the serum.

It has long been recognized that the serum should be injected as soon as possible after the diagnosis of tetanus is made. It is known from our hospital records, that individuals with incipient tetanus have been led by the early and slight stiffness and soreness of the jaw muscles to consult a physician. When the possibility of tetanus was overlooked by the physician, two or three or more days passed before the symptoms reached that stage at which no one could miss the diagnosis. The difference of a single day or even of a few hours in the time of administration of antitetanic serum may mean the difference between life and death. That this is true is clearly shown by our experiments with dogs and monkeys.

The time factor is particularly important in those cases in which the toxin is being steadily but not too rapidly elaborated, because

we have here a chance of intervening with our serum before a full lethal dose has been fixed upon the tissues.

SUMMARY

1. The results of our experiments, taken with a critical study of case histories of human tetanus, have led us to conclude that antitetanic serum is powerless to mitigate or to abolish existing and clearly evident symptoms of a descending tetanus in animals and in human beings, whose tissues had fixed one or more lethal doses of the toxin before the serum was used. Under these circumstances the serum fails to save life, and it cannot therefore be thought of as having a specific, curative action.

2. We have, however, found, contrary to the usual belief, that tetanic animals, inclusive of the highly susceptible monkey, *whose tissues have fixed many lethal doses of the toxin*, can be kept from showing any signs of tetanus if the serum is given not later than at a certain stage of the incubation period. After the injection of from one to 3 lethal doses into these animals, the time interval within which we can intervene with serum to save life may be as long as 40 hours. After the injection of 10 or more lethal doses, this interval is reduced to 24 hours at best, even when excessive amounts of the serum are used. In all instances, however, provided that one or more lethal doses of the toxin have been fixed, the serum fails to save life if its injection be delayed until *the incubation period merges into the period of onset of symptoms*, that is to say, until unmistakable symptoms of a descending or general tetanus have appeared. The results of our work with animals that had been injected with many lethal doses of the toxin testify to the remarkable prophylactic effect of the timely injection of adequate amounts of the serum.

3. There are many instances of incipient human tetanus the nature of which justifies the hope of a favorable outcome following injection of the serum. These are of that large class of cases in which somewhat less than a fatal dose of the toxin, let us say three quarters, has been fixed in the body, and in which pronounced symptoms of a descending tetanus have accordingly appeared, at the time the patient is first seen. In a case of human tetanus we cannot know whether a full lethal dose, or one that allows of recovery, has already been fixed

at the time of admission, even though the presence of the symptoms points to the fixation of a full lethal dose. Many cases of this kind reported in the pre-serum days recovered because the total amount of toxin produced in the infected wound fell short of this dose. Another class with an apparently equal chance of surviving, as judged by the initial symptoms, at the time of admission, failed to do so for the undoubted reason that a full lethal dose of the toxin was finally fixed in the body in consequence of a further elaboration of the toxin in the infected wound. A certain but unknown number of cases of this type, that would later have succumbed to the fatal action of such further increments of the toxin from the infected wound, are now being saved by the injection of liberal amounts of the serum, and this is possible only because they are protected by the circulating antitoxin of the serum from the fatal action of additional amounts of newly formed toxin.

4. There is another class of cases, the nature of which is such as to induce an even more hopeful state of mind in respect to the extraordinary life-saving action of liberal amounts of the serum. This class includes every type of a prior local tetanus in which the tissues of the individual have not yet fixed, but will eventually fix a fatal amount of the toxin, but in which no symptoms consequent to the fixation of a full lethal dose have yet appeared. In earlier days, cases of this kind always terminated fatally. These patients can now be saved, provided that a liberal amount of the antitoxin is injected as soon as the local tetanus is recognized, and before definite symptoms of a descending tetanus have appeared. This opinion is supported by the results of experiments with dogs and monkeys, in which we have the advantage of being able to determine in advance the neutralizing capacity of antitetanic serum for varying amounts of fixed toxin and at varying intervals after its administration.

5. The belief that antitetanic serum is entirely devoid of curative action in the true sense of the term, as defined in (1), does not absolve us from the obligation of making the fullest use of its extraordinary prophylactic action. Because of our ignorance in respect to the factors involved in the earliest stages of human tetanus, and particularly, whether the toxin is being slowly, or more rapidly, elaborated at the site of infection, or in such large amounts as to furnish many

lethal doses, we must continue to employ antitetanic serum in all cases of the disease, at the earliest possible moment, in order to save that limited number that we have every reason to believe can be saved.

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BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

MacLeod's Physiology in Modern Medicine. 8th Ed. Edited by PHILIP BARD. 1051 pp. Illus. $8.50. (*C. V. Mosby Company, St. Louis, 1938.*)

The 7th edition of MacLeod's well known physiology appeared in 1935 shortly before the lamented death of the author. The present 8th edition is a new book, or practically a new book. While it follows to some extent the arrangement of subjects in former editions substantial changes have been made, and the book has been largely rewritten by a group of physiologists under the editorship of Professor Bard of the Johns Hopkins University. Dr. Bard contributes a very interesting preface defining the scope and spirit of the book, and is responsible for Part I on the Neuromuscular and Central Nervous System, as well as the chapter on Regulation of the Body Temperature. Part II on The Special Senses is written by Dr. J. M. D. Olmsted of the University of California; Part III on The Circulation by Dr. H. C. Bazett of the University of Pennsylvania; Part IV on Respiration by Dr. C. F. Schmidt of the University of Pennsylvania; Part V on The Physiology of the Alimentary Tract by Dr. G. R. Cowgill of Yale University; Part VI on Metabolism and Nutrition by Dr. C. L. Gemmill of the Johns Hopkins University; Part VII on The Endocrine Glands by Dr. R. G. Hoskins of the Memorial Foundation for Neuro-endocrine Research and Harvard University; Part VIII on The Distribution and Regulation of Water in the Body and Part IX on The Kidney by Dr. M. I. Gregersen of Columbia University, and the chapter on The Coagulation of the Blood by Dr. Harry Eagle of the U. S. P. H. S. and Johns Hopkins University. The book is, therefore, the product of a collaboration by nine American writers each of whom is well known for his scientific work in the field to which he contributes.

It may be said at once that the book is excellently well done. The fact that each author writes upon a part of the subject with the literature of which he is familiar gives it an authoritative character, such as can scarcely be claimed for a text-book prepared by a single author. On the other hand, coöperative authorship suggests the possibility that certain in-between subjects may be incompletely covered, and, indeed, examination of the contents indicates that some topics have been dealt with quite briefly. Doubtless, this point was well considered by the Editor. The title, *Physiology in Modern Medicine*, implies that emphasis is meant to be placed on those parts of physiology that at present are most directly applicable to medicine.

The book is written to the level of the special student of the subject. Workers in physiology and physicians interested in special phases will find it most helpful

in presenting the present day status of physiological science. There may be some question whether the medical student who is not expecting to go into research is so well served. The subject is tremendously complex and he may find it pretty heavy going to follow so many details of procedure and discussion. But the modern student comes to his medical work after a serious discipline in physics and chemistry, and probably he is well prepared to do his physiology in the same rigorous fashion.

W. H. H.

Crippled Children. Their Treatment and Orthopedic Nursing. By EARL D. MCBRIDE and WINIFRED R. SINK. 2nd ed. 379 pp. Illus. $3.50. (*C. V. Mosby Co., St. Louis, 1937.*)

This second edition of a book which has proved of such distinct value to nurses, medical social workers and some parents, has been much improved by elaboration of chapters previously treated very briefly, by the addition of photographs, and by the insertion of material developed by a practically experienced nurse.

The introductory chapter might well be used as the catechism for all who are in contact with handicapped people, especially children. The lack of a proper "orthopedic attitude" and of a patient and pleasant personality might readily neutralize the results of years of training.

In relatively simple language with a minimum of technical terms, the author has summarized all of the important diseases of the bone and joint system and has presented a general, common-sense viewpoint toward them, rather than a too narrow theoretical or personal one.

The chapter on Physical Therapy reviews practically all the commonly used methods and offers a relatively simple explanation of the fundamental features of each. The emphatic statements, that harm can be done by the incorrect selection and use of the modalities, and that a physician who understands the principles of each apparatus as well as its proper and improper effects must supervise all physical therapy, could well have been put in large black type. Too often a machine is bought and used upon the basis of statements by a salesman totally ignorant of its limitations and possible dangers. Physical Therapy never should be given without the consent and advice of a competently trained and responsible physician.

The chapter on braces is clear and simple with sufficient text to allow a nurse to appreciate how braces should look when properly made.

Occupational therapy has been only very briefly dealt with and this topic will no doubt be covered more fully in a later edition. Since the crippled child is now considered to be of any age up to 21 years, the fields of occupational and vocational therapy are becoming more widely used and much more developed. The steps from early treatment medically through physical therapy, occupational therapy, vocational training, and placement in industry are now inseparably associated and must be blended with the social aspects, so that the individual may assume the position in his community that will be the most useful and

economically advantageous to himself, his family and his associates, consistent with his physical abilities. The appendix lists a well selected number of words commonly used and gives each a short definition. This section will no doubt be constantly referred to by all.

The book fills a place in the field of orthopedics that has been only lightly treated heretofore. The author and his collaborator are to be commended for covering so briefly and so thoroughly such a varied and complicated branch of medical work. The book appears at a time when it is most needed.

A. F. V.

Les Courants de haute fréquence. Applications de tension (Courants de résonance). Par Le Docteur GEORGES RONNEAUX. 156 pp. Illus. Paper, 30 fr. (*Paris. Gauthier-Villars, 1937.*)

This small volume of 156 pages is one of an extensive series published under the heading of Les Actualités Physiothérapiques under the direction of Dr. Duhem who wrote several of the works himself.

Under the heading of *Electrothérapie* alone there are eight volumes of which this one is the sixth. It deals with an electrical modality that is comparatively little used in this country at present, but the one from which the conventional diathermy and the more recent short wave diathermy have developed. The author laments that the currents of high tension have been largely superseded in medicine by diathermy, for he is convinced that their physiological effects are not the same.

The book contains a brief historical introduction, a consideration of the physics of these currents, a discussion of their physiological effects and an exposition of the use of the modality in treating pathological conditions. The style is characterized by a mild verbosity and the exposition is none too critical.

T. P. S.

The Cerebrospinal Fluid. By H. HOUSTON MERRITT and FRANK FREMONT-SMITH. 333 pp. Illus. $5.00. (*W. B. Saunders Company, Philadelphia, 1937.*)

The anatomy and physiology, *i.e.*, the source, mode of formation, circulation and function of the cerebrospinal fluid are dealt with very summarily. In the more extensive chapter on the physical properties and chemical constituents of the cerebrospinal fluid in normal and pathological conditions, the treatment of the individual items also suffers from the lack of a comprehensive discussion.

The description of the technic of cisternal and lumbar puncture and the routine examination of the cerebrospinal fluid will be of aid to the clinician not initiated in the routine laboratory work. He will find there methods for the cell count, determination of protein, sugar, chloride, nonprotein nitrogen, urea, Ca, bromide, colloidal gold test and bacteriologic examination. In discussing the quantitative determination of bromide the authors refer only to their modification of the

method of Toxopeus, and regarding their own procedure as "satisfactory," they find it unnecessary to scrutinize the experience of other workers in this field.

The chapter on roentgenography of the ventriculo-subarachnoid space gives a concise but satisfactory description of the various available methods.

A considerable portion—nearly half—of the book deals with cerebrospinal fluid syndromes in a very wide range of diseases. Here, as elsewhere, a more comprehensive use of bibliographical data, and not merely references to names of authors, would add greatly to the value of the authors' large material. As it stands, however, the book will prove to be of assistance to the clinician.

S. K.

The Practice of Medicine. By JONATHAN CAMPBELL MEAKINS. 1343 pp. Illus. $10.00. (*C. V. Mosby Company, St. Louis, 1936.*)

A truly monumental volume. It epitomizes to date in more than 1300 pages the well spent scientific life of the author as physiologist and physician. He is ably supported in special departments by collaborators: by E. H. Mason, in diseases of metabolism and the ductless glands; Walter de M. Scriver, in diseases of the urinary system; and J. N. Petersen, in diseases of the nervous system. The book is more than just another practice of medicine. It is a combination of applied physiology and practical medicine, for which the author is eminently fitted. There are many fine illustrations, some of them colored. The first thought of the reviewer was similar to that of Dr. Johnson, relating to a dog walking on his hind legs—not to consider how it might be done better, but to marvel that it had been done at all. The book, however, is an outstanding treatise and may be highly recommended.

A few criticisms can always be made, and in this case the physiological approach on the part of the author exposes him to a certain danger, namely, too subtle thought. Thus, he emphasizes vasomotor disturbances in pulmonary embolism, the symptoms of which would seem, on the basis of common sense, attributable directly to cardiac embarrassment. Even in a gross mechanical disturbance, such as coarctation of the aorta, a peripheral vasoconstriction is assumed. The author holds to the popular view that "essential" hypertension in its early stages is due undoubtedly to a vasopressor or vascular hypertonic effect situated in the general arterioles, yet wisely refrains from advocating any of the surgical therapeutic efforts to relieve hypertension. There are some, including the reviewer, who consider so-called essential hypertension most likely an organic disease from the start; and look upon all efforts at reducing blood pressure artificially as unwise. As long as a physiological explanation of hypertension is generally advocated, the surgeon can hardly be blamed for his efforts to reduce pressure by almost any method. The reviewer is inclined to regret the rather broad application of the term pectoriloquy in this book as well as in modern usage. It would probably be better to limit its use to what is actually heard over a cavity, and be in accord with Laennec. The author does not mention the use of iodides in chronic bron-

chitis. This form of therapy has proved so helpful to many patients, that a word must be said here in its support.

Only one major mistake has been found. On page 357, beginning with the premise that 1 cc. is the equivalent of 10 minims, the author calculates a typical course of digitalis therapy and arrives at a dose exactly two-thirds of the correct one. Fortunately, the error is on the side of conservatism, but it is so important that it should be corrected in the next printing. With these possible limitations, it may be said that the book is a real addition to medical literature and will form a valuable part of the practitioner's library.

J. T. K.

Alcohol. One Man's Meat—. By EDWARD A. STRECKER and FRANCIS T. CHAMBERS, JR. 230 pp. $2.50. (*The Macmillan Company, New York, 1938.*)

This book gives the alcoholic, his relatives and the physician a sympathetic picture of the general problem of the pathological drinker. The alcoholic is shown as one who has sought release from personality difficulties. His personality type and the common varieties of problems with which he is faced are clearly defined. The section on treatment, beginning with the first interview with such a patient, gives a full account of a common-sense type of management based on no particular theories, but aimed to take care of all aspects of the individual's functioning.

The book is remarkably sympathetic and constructive in attitude, and can be recommended to anyone interested in the problem and specifically to the drinker who wants real insight into his own condition and an explanation of what modern scientific treatment offers and does. It should convince any drinker that if he means to be cured, he must realize that he can never drink again; that he has an infinite capacity for rationalizing his problem and for projecting the responsibility of it upon others. Any physician might well begin his treatment of an alcoholic patient by giving him this book to read.

T. R.

The Endocrines in Theory and Practice. Articles republished from the British Medical Journal. Pp. ix, 278. $3.50. (*P. Blakiston's Son and Co., Philadelphia, 1937.*)

Many times in the past year medical practitioners have expressed a desire for a book on endocrinology which was conservative, brief, authoritative, up to date and written in simple terminology. Previously I have known of no book to recommend which would fulfill these criteria. "The Endocrines in Theory and Practice", written by a distinguished group of British endocrinologists, does, however, meet this need in admirable fashion.

In subjects so active and rapidly advancing as those dealt with, and especially when covered by so many authors, one might expect to find here and there statements with which one does not entirely agree. But on the whole the articles are so conservative, and are written by men so preeminent in their fields, that this reviewer feels no hesitation in giving the book his highest recommendation.

J. E. H.

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John Whitridge Williams: Academic Aspects and Bibliography. By J. Morris Slemons. Foreword by John C. French. 1935. 121 pages, frontispiece, 12mo, boards, $1.50.

The life of John Whitridge Williams, as Dr. Slemons has told the story, is an essential chapter in the history of American medicine. Dr. Williams shared largely in the far-reaching effect The Johns Hopkins School of Medicine has had on instruction and research and as a brilliant investigator in his own specialty and the author of an authoritative textbook, he advanced scientific knowledge and human welfare. Dr. Slemons had been aware of the human interest of his subject; and by virtue first of intimate association as student and junior colleague and later as scientist and practitioner in the same field thousands of miles away, he has been able to bring to it both warm personal feeling and scholarly detachment. He has performed a congenial task with distinct success.

Papers and Addresses. By William Henry Welch. 1921. Three volumes, 2013 pages, 4to, $20.00.

Dr. Welch's writings are scattered through a great variety of publications, many of which are more or less inaccessible. It was accordingly decided to publish his numerous papers and addresses. These have been collated under the following headings: Volume I. Pathology, Preventive Medicine. Volume II. Bacteriology. Volume III. Medical Education, History, Miscellaneous, Bibliography, Index.

Surgical Papers of William Stewart Halsted. 1928. Two volumes, 1232 pages, illustrated, 4to, $15.00.

When one considers the number, originality and importance of Dr. Halsted's contributions to surgery and their scattered and often not readily accessible form in various journals and reports, it will be realized that the publication of his collected papers not only constitutes a worthy tribute to his memory, but also renders a welcome and much needed service in making available these significant contributions.

William Stewart Halsted: Surgeon. By W. G. MacCallum. 260 pages, 18 illustrations, 8vo, $2.75.

A brief history of the life of the first surgeon of The Johns Hopkins Hospital. Dr. Halsted, who died in 1922, was one of the four men whose names are best known in the establishment of the fame of The Johns Hopkins Medical School and Hospital. He formed a new school of surgery and trained in his own methods and modes of thought many of the men who are now prominent surgeons in this country.

Franklin Paine Mall: The Story of a Mind. By Florence Rena Sabin. 355 pages, 8 illustrations, 8vo, $2.75.

This book seeks to give the story of the development of Mall's mind and its influence on medical science and on medical education. The writer worked for twenty years under Mall and the theory of education outlined in this volume was thus learned directly from him. It is hoped that the new generation of medical students who have never known Mall may gain, through these pages, some idea of how this man has influenced their lives and that, too, they may gain an insight into the difficulties through which the generation that preceded theirs won the privileges they now enjoy. Mall is but little known to the public, but one has only to mention his name to any of his colleagues to sense that he was one of the leaders of his generation.